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Dispersal of American Lobsters, *Homarus americanus*, Tagged Off Southern Nova Scotia

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Campbell, A. 1989. Dispersal of American lobsters, *Homarus americanus*, tagged off southern Nova Scotia. Can. J. Fish. Aquat. Sci. 46: 1842–1844.

Of the 5281 American lobsters (*Homarus americanus*) tagged and released off McNutt Island, South Nova Scotia, during 1983–87, 15.8% were captured within 3 yr of release. Although most immature (99%) and mature (86%) lobsters were caught <18.5 km from release, mature lobsters moved a greater mean distance (25.8 km) than immature lobsters (3.6 km). Of the lobsters that moved > 30 km eight moved south to the continental slope near Browns Bank and 11 moved west of Cape Sable to the inshore areas of southwestern Nova Scotia, the midshore area of German Bank and offshore to Browns Bank and Georges Bank. This and other studies of tagged lobsters indicate that there is some movement of lobsters east and west of the Cape Sable area, the southern tip of Nova Scotia, suggesting some mixing between lobster stocks off eastern Nova Scotia and southwestern Nova Scotia. The long distance movement and mixing of mature lobsters suggests that attempts to use tagging information in locating biological boundaries between lobster stocks in the Gulf of Maine, adjoining Continental Shelf, Scotian Shelf and inshore areas of southwestern Nova Scotia and southern end of eastern Nova Scotia may be a difficult task.

Parmi les 5281 homards d'Amériques (*Homarus americanus*) marqués et relâchés au large de l'île McNutt au sud de la Nouvelle-Écosse, de 1983 à 1987, 15,8 % de ces homards ont été recapturés trois ans après qu'ils aient été relâchés. La plupart des homards immatures (99 %) et la plupart des homards matures (86 %) ont été recapturés à plus de 18,5 km de l'endroit où ils ont été relâchés, mais les homards matures ont parcouru une plus grande distance (25,8 km) que les homards immatures (3,6 km). Parmi les homards qui ont parcouru plus de 30 km, huit homards se sont déplacés vers le talus continental, au sud, près du banc Browns et 11 homards se sont rendus à l'ouest du cap de Sable dans la région côtière du sud-ouest de la Nouvelle-Écosse, les eaux semi-hauturières du banc German et en haute mer sur les bancs Browns et Georges. Cette étude et d'autres études réalisées à l'aide de homards marqués montrent que les homards peuvent se rendre aussi bien à l'est qu'à l'ouest de la région du cap de Sable, situé à la pointe sud de la Nouvelle-Écosse, ce qui laisse penser qu'il pourrait y avoir un certain mélange des stocks de homards au large des côtes est et sud-ouest de la Nouvelle-Écosse. Les déplacements et le mélange des stocks de homards sur de longues distances auraient pour effet de compliquer les essais de localisation des frontières biologiques entre les stocks de homards dans le golfe du Maine, qui est adjacent à la plate-forme continentale, à la plate-forme Scotian et aux régions côtières du sud-ouest de la Nouvelle-Écosse et de l'extrémité sud de l'est de la Nouvelle-Écosse.

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The American lobster (*Homarus americanus*) is one of the most valuable commercial species in the coastal waters of southern Nova Scotia (Campbell and Mohn 1983; Campbell 1989a). Essential to management of this resource is the understanding of the distribution and migration of lobsters in the area. Yet the geographic discreteness of lobster stocks has been debated for a number of years (e.g., Aiken 1971; Wilder 1974; Stasko 1978; Campbell and Mohn 1983; Pezzack 1987a). Recent tagging studies indicated some mixing between lobsters of southwestern Nova Scotia, the Gulf of Maine and adjoining Continental Shelf (Campbell and Stasko 1985). Based on the lack of tag recoveries, Pezzack (1987a) suggested that the separation boundary between two lobster stocks of southwestern Nova Scotia and the southeast coast of Nova Scotia was possibly in the Cape Sable area of the southern tip of Nova Scotia. Indeed this approximate demarcation between the two lobster stocks was generally accepted for management purposes (Pringle et al. 1983). Yet little biological information has been available to indicate the extent of intermixing between lobsters from these two geographic areas. The main objective of the present study was to determine if there has been lobster movement between the east and west side of the Cape Sable area off southern Nova Scotia. Recent ongoing tagging studies (e.g., Campbell 1989b) have confirmed much of the findings of Campbell and Stasko (1985, 1986) and the present study highlights the new information on lobster movements not previously recorded.

Materials and Methods

A total of 5281 lobsters ranging from 20 to 172 mm CL (carapace length, measured from the base of the eye socket to the posterior margin of the carapace parallel to the mid-dorsal line) was tagged and released off McNutt Island (43°37′N,

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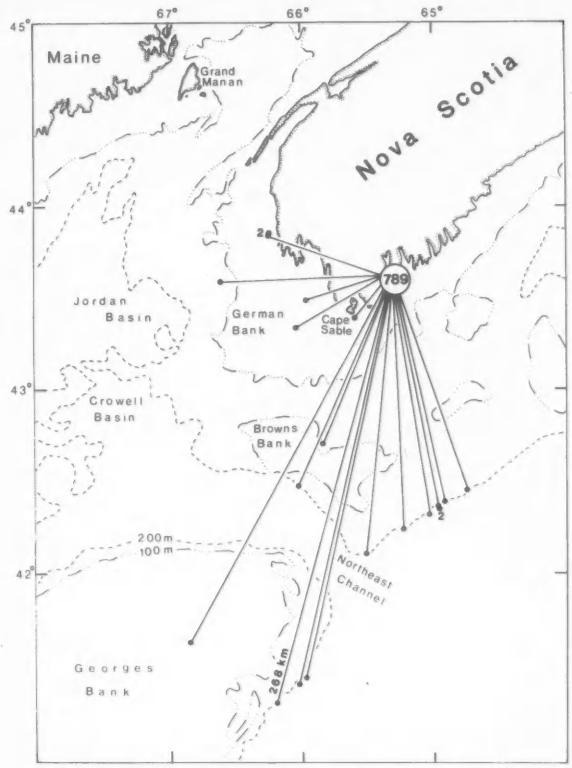

FIG. 1. Recapture points and straight-line distances moved >30 km for tagged lobsters released near McNutt Island. Each dot represents one recapture, except where a group of dots has a numeral indicating the number of recaptures; 789 lobsters were recaptured within 30 km of the release area.

67°17′W) near Shelburne, southern Nova Scotia during 21 July–22 August 1985, 1986, and 1987. Trap-caught lobsters were tagged and released within 10 min of removal from the water and within 0.5 km of the capture site at a mean depth of 7.0 m (range ±5.0 m). Each lobster was tagged with a sphyrion anchor tag (Scarratt 1970) inserted with a hypodermic needle into the dorsal musculature between the carapace and abdomen of the lobster, with the coded yellow tubing projecting dorsally. Lobsters at release were categorized as male, female, and as immature (<95 mm CL) and mature (≥95 mm CL) based on the median size at physiological maturity (Campbell 1983) estimated with the ovary examination technique (Aiken and Waddy 1980). Of the total released, 6.5% were mature lobsters. Release depth, location, date, CL, sex and tag number of each lobster were recorded. A reward of up to $5 was paid for tag returns with information on recapture date, location, depth and CL. Data from tag returns up to 31 March 1988 were analyzed in this study.

Straight-line distances and direction travelled were calculated from the latitude and longitude of release and recapture locations (Campbell et al. 1983). Mean distances moved between size groups and sexes were compared by a one-way ANOVA and Student-Neuman-Keuls test (Kim and Kohout 1975) after transforming distance moved data by $\log_{10}(x+1)$. Since for each size group there were no significant differences ($P > 0.05$) between males and females regarding time at liberty and distance travelled, data for both sexes were combined according to each size group.

Interpretation of movement data obtained from the recovery of tagged lobsters provided by commercial fishermen should be considered with caution because bias can be introduced by the nonuniform temporal and spatial distribution of fishing effort (see discussions by Fogarty et al. 1980; Bennett and Brown 1983; Campbell and Stasko 1985; Pezzack 1987a). The opening fishing season in the inshore areas was from the third Tuesday in November to the 31 May, and most inshore tags were recaptured during periods of high fishing effort during the fall (November and December) and spring (April and May), whereas tags recaptured offshore (>93 km from the coast) were returned year round. The distribution of traps should reflect the location and trapability of lobsters in the area. Fishermen usually concentrate their fishing effort in areas of high productivity where lobster densities are high, but they may also set exploratory traps in areas that are marginally profitable. Although the rate of tag loss during molting and/or mortality due to poor tag application was unknown, individuals applying tags to lobsters were well trained and experienced so that tag loss was considered minimal. However, Ennis (1986) found tag loss ranged between 24–36% for lobsters tagged in Newfoundland. The straight-line distance travelled between release and recapture points can be misleading since some lobsters may undergo seasonal long-distance, deep-shallow migrations, returning close to the original release areas (Campbell 1986; Pezzack and Duggan 1986). The effectiveness of the tag return system is difficult to evaluate and some recaptured lobsters were probably not reported. There is some doubt whether all fishermen reported tag recaptures since there has been conflict between fishermen groups concerning various management regulations (Campbell 1989a). The percentage of tags returned in this study (15.8%) is comparable with those reported by Campbell and Stasko (1985) but lower than for other areas (Campbell and Stasko 1986; Pezzack 1987a). Despite the possible shortcomings of the tag return system, some important information on the movement of lobsters off southern Nova Scotia was obtained.

Results

Of the 5281 lobsters tagged, 15.8% were recaptured, but only 808 lobsters had known recapture locations that could be used for analyses. Most (86.8%) lobsters were recaptured within a year of release (mean time at liberty was 5.0 mo) with three lobsters caught 30 mo after release. Most immature (99.0%, $N = 706$) and mature (86.3%, $N = 102$) lobsters were caught <18.5 km from the release sites; 0.1% immature and 12.7% mature lobsters moved >92.6 km. Mature lobsters moved a greater ($P < 0.01$) mean distance (25.8 km) than immature lobsters (3.6 km). The maximum distance travelled was 268 km (Fig. 1). Inspection of the straight-line tracks between release and recapture points of the few ($N = 19$) lobsters that moved >30 km indicated some exchange of lobsters between inshore and offshore areas off southern Nova Scotia (Fig. 1). Some lobsters moved from the inshore areas of south Nova Scotia in a southerly direction to the continental slope near Browns Bank. A total of 11 lobsters moved west of the north–south line at Cape Sable to inshore areas of southwestern Nova Scotia, the midshore area of German Bank and offshore to Browns Bank and Georges Bank (Fig. 1).

Discussion

The main new result of this study is that there was some movement of tagged lobsters from east to west areas of Cape Sable, off the southern tip of Nova Scotia. Other results confirm the findings of (Campbell and Stasko 1985, 1986) that: (1) mature lobsters moved farther than immature lobsters; and (2) the long-distance movement of some mature lobsters allowed mixing with other lobsters throughout inshore and offshore areas in the Gulf of Maine and the adjoining continental shelf.

The movement of some tagged lobsters between east and west (including southerly dispersal) off Cape Sable suggests that this area is probably not a biological boundary of lobster stocks between southwestern Nova Scotia and eastern Nova Scotia as suggested by Pezzack (1987a). Examination of other tagging studies (Campbell and Stasko 1985, fig. 5, 7; Campbell and Stasko 1986, fig. 7; Pezzack 1987a, fig. 3, 4; Campbell 1989b, fig. 4, 6) indicate some interchange of tagged lobsters in this area (albeit from only a few tag returns). The general southwesterly direction of the long-distance movements of lobsters may be due to the physiographic constraints imposed by the coastline of Nova Scotia. However, with the lack of a midshore and offshore fishery off eastern Nova Scotia and the Scotian Shelf (Pezzack 1983, 1984, 1987b,) and consequent lack of potential tag returns, the extent of lobster movements in this area is difficult to determine. Thus, the use of tagging methods to determine the exact biological boundary, if there is one, between these two stocks is probably, at present, impractical. Other methods such as analysis of landing trends (Campbell and Mohn 1983; Harding et al. 1983), morphometrics (Campbell and Mohn 1982) and examination of isozymes of muscle tissue (Odense and Annand 1978) of lobsters also indicate some overlap between lobster stocks from southwestern and eastern Nova Scotia. Therefore, the estimation of a boundary between these two lobster stocks near Cape Sable should at present be considered mainly for stock management purposes of socioeconomic convenience, rather than for separation of two biologically distinct lobster populations.

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A Model of Feeding by Parasitic Lampreys

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Cochran, P. A., and J. F. Kitchell. 1989. A model of feeding by parasitic lampreys. Can. J. Fish. Aquat. Sci. 46: 1845–1852.

We derived a quantitative feeding model to estimate energy intake by parasitic sea lampreys (*Petromyzon marinus*) as a function of lamprey mass, host mass, and attachment time. Comparison of model predictions with observed growth by lampreys with known feeding histories suggests that the feeding model, used in conjunction with a previous model of lamprey energetics, generates unbiased predictions of energy intake and subsequent growth. On the basis of the relationship between energy intake and attachment time, and in accordance with previous theory on partial prey consumption, we hypothesized that duration of attachment by lampreys to individual hosts should vary inversely with host density. No significant difference in attachment time or latency to attack at two host densities could be detected, although the first attachment by a lamprey tended to be longer than its second, regardless of host density.

Les auteurs ont élaboré un modèle d'alimentation quantitative visant à calculer l'apport énergétique des lamproies de mer parasites (*Petromyzon marinus*) en fonction de la masse des lamproies, de la masse de l'hôte et de la durée de fixation. La comparaison des prédictions obtenues et de la croissance observée chez les lamproies dont l'alimentation est connue semble indiquer que ce modèle, utilisé avec un modèle de l'énergétique des lamproies élaboré précédemment, permet d'obtenir des prédictions non biaisées de l'apport énergétique et de la croissance qui en résulte. Conformément à une théorie précédente portant sur la consommation partielle des proies, et parce qu'il existe un lien entre l'apport énergétique et la durée de fixation, les auteurs ont émis l'hypothèse suivante : la durée de la fixation des lamproies à leur hôte est inversement proportionnelle à la densité des hôtes. Peu importe la densité des hôtes, aucune différence significative dans la durée de fixation ni dans le temps écoulé avant la fixation n'a pu être observée, bien que la première fixation d'une lamproie ait tendance à être plus longue que la deuxième.

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One puzzling aspect of interactions between populations of parasitic lampreys and their hosts has been the remarkable differences in host mortality that have been observed among localities. The evidence for such differences among freshwater populations of the sea lamprey (*Petromyzon marinus*) has been summarized by Kitchell and Breck (1980); lamprey-induced mortality is significant over nearly two orders of magnitude of variation in scarring frequency [the use of injuries to make inferences about the impact of predation has been addressed in other contexts by Schoener (1979), Murtaugh (1981), and Morin (1985)]. Although some host populations persist even when almost all fish carry multiple scars (Pearce et al. 1980), experimental work summarized by Farmer (1980) suggests that an adult lamprey can kill even large salmonids in a relatively short time, and lamprey-induced mortality rates may be high even though observed rates of wounding and scarring are low (Smith et al. 1974). The devastating contribution of sea lampreys to the collapse of lake trout (*Salvelinus namaycush*) and other fish stocks in the Upper Great Lakes is well known (Smith 1971), yet landlocked sea lamprey may have coexisted with lake trout and other fishes in lakes such as Lake Ontario, Lake Champlain, and the Finger Lakes for hundreds if not thousands of years (Brussard et al. 1981).

Kitchell and Breck (1980) proposed a hypothesis to explain the apparently conflicting relationships among lamprey-induced mortality and marking rates: lampreys may exercise choice between acting as predators, which kill their prey, and parasites, which do not, based on their perception of host density. They suggested that selection should favor parasitic feeding when host densities are high because (a) host blood quality declines as a lamprey remains attached to a single host (Farmer 1980), and (b) blood is a rapidly renewable resource, and its level will be restored after a lamprey detaches. It is perhaps more parsimonious to consider (b) a fortuitous consequence rather than a selective advantage, since the latter could only result from group selection.

A hypothesis closely related to that of Kitchell and Breck (1980), but based on duration of attachment, can be adapted from the theory of optimal foraging as applied to the partial consumption of prey (Cook and Cockrell 1978; Sih 1980). If quality of food ingested declines as a function of time spent feeding on an individual prey item, this theory predicts that feeding time should be positively related to expected costs (time and energy) of searching for another item. Thus, feeding time should decline when prey densities and encounter rates are high

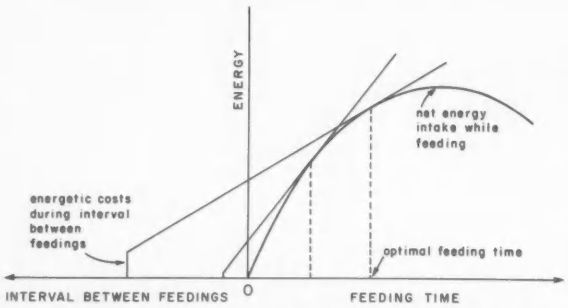

FIG. 1. Net energy intake by a predator from a single prey item as a function of time spent feeding on the item. The tangent drawn from an interval between feedings on the x axis locates the corresponding optimal feeding time in terms of overall rate of net energy intake. Optimal feeding time increases as either intercatch interval or non-feeding costs increase. Adapted from Sih (1980).

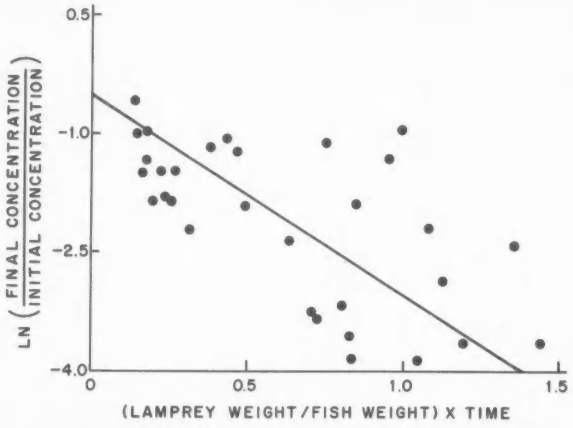

FIG. 2. Scatterplot of the ratio of the final to initial concentration of ^{51}Cr in blood of host trout (B/B_0) versus the product of the ratio of lamprey to host wet weight (L/F) and feeding time in days (t). The functional regression is $\ln(B/B_0) = -0.50–2.54\ (L/F)t$, ($r^2 = 0.39$). Data from Farmer (1974).

(Fig. 1). Food quality is most often measured in terms of energy (Pyke et al. 1977). Mean blood energy concentration (kJ/g wet weight) of trout has been shown to have declined by 84% just prior to death from lamprey attacks (Farmer 1980).

Implications of this hypothesis for lamprey–host dynamics are essentially the same as those outlined by Kitchell and Breck (1980). All else equal, a shorter mean attachment time generally should be associated with a lower host mortality rate. (However, if for some range of attachment times, stress, infection by pathogens, or some other agent causes delayed mortality subsequent to lamprey detachment, then a decrease in mean attachment time might result in a greater host mortality rate because more fish will be attacked). A lamprey–host system conceivably could be characterized by two stable states: a lower equilibrium with the host population held in check by high lamprey-induced mortality rates as a result of long attachment times, and a higher equilibrium with very short attachment times and low host mortality.

By far the greatest bulk of fisheries research on the trophic impact of parasitic phase sea lamprey has involved measurement and analysis of population attributes (marking rates, mortality rates, etc.). However, the impact of a lamprey population is the result of individual behaviors, and natural selection operates most strongly at the level of the individual. With this paper we address the need for informed observation and analysis of individual lamprey behavior. We develop and evaluate a quantitative model of energy intake by a lamprey from its host. We describe a laboratory experiment on the effect of host density on lamprey attachment times. Finally, we show how the lamprey feeding model can be used to examine such phenomena as host size selectivity.

Methods and Materials

The Feeding Model

Our model is applicable to those parasitic lampreys, such as *P. marinus*, that feed by removing blood through a hole rasped in the side of a fish (Potter and Hilliard 1987). Assume that the fraction of a host's blood volume removed during any instant by a feeding lamprey is proportional to the biomass of the lamprey (L) and inversely proportional to the biomass of the host (F), although a curvilinear relationship may prove to be more realistic over broader ranges of lamprey and host sizes than those considered here. Assume further that host blood volume remains constant due to osmotic influx of water (Farmer et al. 1975). It follows that

$$(1)\quad dB/dt = -K(L/F)B$$

$$(2)\quad B = B_0\exp(-K(L/F)t)$$

and, by integration,

$$(3)\quad \ln(B/B_0) = -K(L/F)t$$

where K is a proportionality constant, B is the concentration of original blood remaining at time t after the onset of feeding, and B_0 is blood concentration at $t = 0$, the onset of feeding. We tested this hypothesis with data collected by Farmer (1974), who tagged the blood of trout with radioactive ^{51}Cr and measured its decline after feeding by lampreys of known size at 10°C (Fig. 2). The variables in Fig. 2 are strongly correlated ($r = -0.629$, $P<0.01$) and a fit of equation (3) to the data produced a slope (K) of magnitude 2.54. [Probability levels and correlation coefficients presented here are those associated with normal least squares regression, but the GM functional regression advocated by Ricker (1973) was used to obtain model parameters.] The product of this slope and the ratio of lamprey to host biomass provides an estimate of the proportion of the host's blood volume removed daily.

Loss of blood to a feeding lamprey is offset to some extent by blood regeneration. G. Farmer (Dep. Fisheries and Oceans, Halifax, N.S., pers. comm.) observed that blood quality of attacked trout remained similar to that of control fish for some time after the initiation of an attack; his data (Fig. 3) can be used to estimate the minimum length of this interval as a function of the percentage of host blood volume removed daily (V):

$$(4)\quad \ln(Q) = 4.92 - 1.08(\ln(V))$$

where Q is the feeding time in days ($r^2 = 0.759$, $t = -4.69$, $P<0.005$). The feeding time that results in host death (D) can also be related to the percentage of host blood volume removed daily (Fig. 3; see also Farmer et al. 1975):

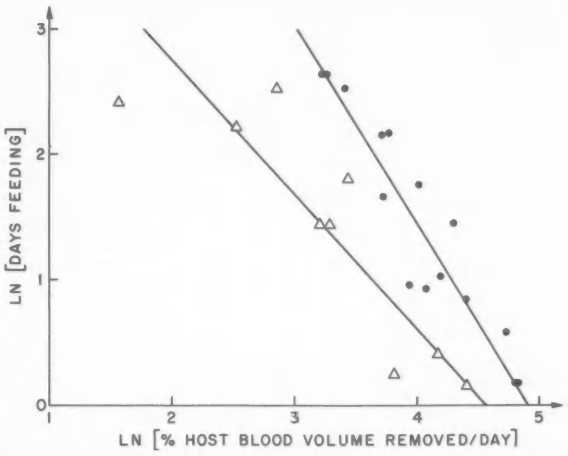

FIG. 3. Scatterplot of feeding time in days versus percentage of host blood volume removed per day for trout with normal blood quality (triangles) and moribund trout with reduced blood quality (dots), with corresponding functional regression lines [text equations (4) and (5), respectively]. Data from Farmer et al. (1975) and G. Farmer (Dep. Fisheries and Oceans, Halifax, N.S., per comm.).

$$(5)\quad \ln(D) = 8.03 - 1.63(\ln(V))$$

$$(r^2 = 0.880,\ t = -9.77,\ P<0.001).$$

One independent data set is consistent with equation (4). Kinnunen and Johnson (1985) allowed sea lampreys with a mean body mass of 13.5 g to feed on rainbow trout with a mean body mass of 309.1 g for 10 d at 12°C. At the end of this period, blood hematocrit and hemoglobin levels had not declined significantly from initial values. According to equation (3) above, the lampreys were removing 11.1% of their hosts' blood volume daily, and according to equation (4), the hosts should have been able to maintain blood quality for 10.2 d. Kinnunen and Johnson (1985) also allowed lampreys with a mean body mass of 25.3 g to feed on trout with a mean body mass of 328.4 g for 8 d, at which time blood hematocrit and hemoglobin had declined significantly. According to equation (3) the lampreys were removing 19.6% of their hosts' blood volume daily, and the hosts should have been able to maintain blood quality for 5.5 d.

The implications of equations (2), (4), and (5) taken together are that a lamprey of a given biomass (L) attached to a trout of a given biomass (F) receives a constant ration for Q days, during which time blood regeneration by the host is sufficient to offset losses to the lamprey (Fig. 4). Daily ration is the product of the proportion of host blood volume removed daily, the blood volume of the host (4.7% of wet body weight for *Oncorhynchus mykiss* (formally *Salmo gairdneri*) in freshwater — Smith 1966), and the energy concentration of the blood (3.29 kJ/g wet weight — Farmer 1980). Proportion of host blood volume removed daily can be estimated in two ways: (a) a posteriori from equation (5) if the host has been killed after a known period of time, or (b) a priori from equation (3) ((i.e., $V/100 = 2.54(L/F)$). Equation (3) implies that daily ration in terms of wet weight is a constant proportion of lamprey biomass ($2.54 \times (L/F) \times 0.047F = 0.119L$). While this may be true on average, individual variability is great (Fig. 2), and the use of equation (5) for individual cases is preferable when applicable.

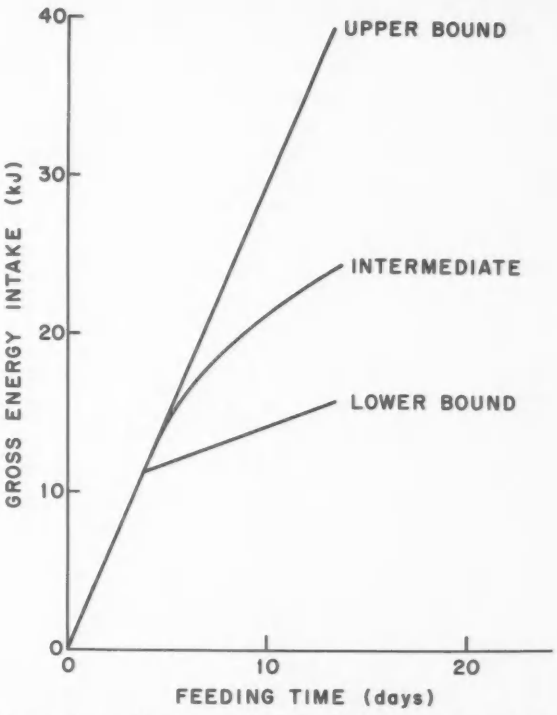

FIG. 4. Predicted gross energy intake by a 7.4 g lamprey from a 68.2 g trout as a function of feeding time. Host death occurs on the fourteenth day of feeding. Upper and lower bound: host blood energy concentration assumed constant at 3.29 and 0.54 kJ/g wet weight, respectively. Intermediate: host blood energy concentration assumed to decline exponentially from 3.29 to 0.54 kJ/g wet weight. Lamprey and host biomass correspond to mean values from first round of experiment.

During the period of feeding from time Q to time D, the predicted point of host death, daily ration declines as host blood energy concentration decreases from 3.29 to 0.53 kJ/g wet weight (Farmer 1980). Although we have no data on the dynamics of this decline, we suggest two extremes that bracket the range of possibilities. At one extreme, it can be assumed that the host's blood regenerative capability continues unabated up to the point of death, so that blood energy level remains constant. This should lead to an overestimate of energy intake. At the other extreme, it can be assumed that blood energy level on day Q declines instantly from 3.29 to 0.53 kJ/g and remains at that level until day D. This should provide a lower bound to energy intake during this interval. A simple and logical intermediate possibility is that blood energy declines exponentially during the period from Q to D (Fig. 4).

The lamprey feeding model thus requires the following information to estimate the energy taken up by a lamprey from its host: the biomass of the lamprey, the biomass of the host, and the duration of attachment. As a preliminary test of the model, we estimated energy consumption by 14 lampreys during the laboratory experiment described below, based on their known feeding histories during the experiment. We did not have direct measurements of the amount of energy actually consumed by each lamprey, but we did measure the weight change experienced by each individual. We therefore used our model estimates of energy uptake in conjunction with the sea lamprey

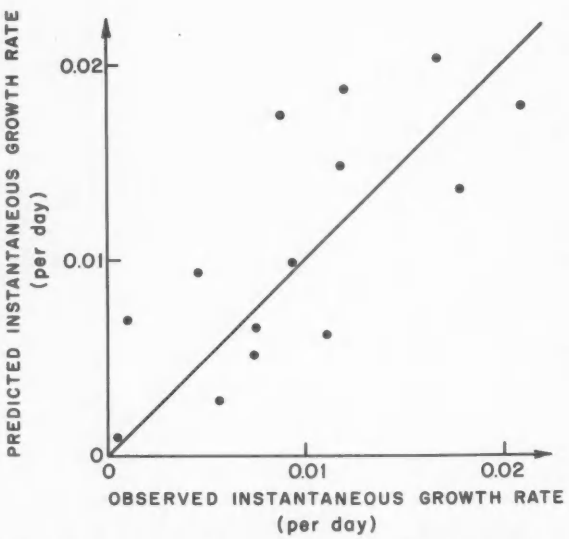

FIG. 5. Predicted instantaneous growth rates (per dayu) of individual experimental lampreys under intermediate assumption of exponential host blood energy decline versus observed rates ($r = 0.75$). The diagonal identity line ($y = x$) is provided for comparison.

bioenergetics model of Kitchell and Breck (1980) to predict growth by each lamprey during the experiment. An upper and lower estimate were provided for each lamprey by using the upper and lower estimates of energy uptake during the period of host blood decline, with the anticipation that these estimates would bracket observed growth. We also estimated growth based on the assumption that blood energy concentration declines exponentially.

Laboratory Experiment

Recently metamorphosed sea lampreys from the Lake Champlain drainage were acquired in December 1982, and maintained in a Living Stream (Frigid Units, Inc., Toledo, OH) filled with 520 L of filtered recirculated water. Photoperiod was held constant at 12:12 h light:dark, and water temperature was maintained at 10 ± 0.5 C. We used small lampreys (5.19–11.29 g wet biomass) so that the experiment could be scaled to a laboratory setting and to minimize individual variation in past feeding history. Small (47–95 g) captive-reared rainbow trout (*Oncorhynchus mykiss*) served as hosts. We chose small hosts so that host blood quality would be quickly affected by lamprey feeding. Lamprey to host biomass ratios ranged from 0.07 to 0.21.

Lampreys were tested individually in 50 × 55 × 38 cm visually isolated compartments of Living Streams. Each compartment contained two bricks that were needed to prevent lampreys from escaping beneath the false bottom of the tank. These bricks also provided some cover for the trout and minimized agonistic interactions when more than one trout were present.

We compared attachment time and latency to attack for individual lampreys when confined with one and two rainbow trout. Thus, attachment time was measured for each lamprey in the presence and absence of an alternative host. Latency to attack, the interval between the beginning of a treatment and attachment by the lamprey to its host, may reflect both the availability of hosts and the motivation of a lamprey to feed; we had no way of separating these factors, but expected that, on average, latency to attack would be greater when only one host was available. To control for the possible effect of treatment order, seven lampreys were initially confined with one trout and seven lampreys were initially confined with two trout. An attempt was made to minimize variation across treatments in the size of trout to which each lamprey was exposed; the mean ratio of the biomass of the larger to smaller host attacked by an individual lamprey during the experiment was 1.06 (range: 1.00–1.22). Observations were made at intervals of at most 12 h. Only attachments that resulted in a wound were included in the analysis. Lampreys were anesthetized with tricaine methanesulfonate and weighed prior to the experiment and subsequent to their final detachments. To allow for gut clearance, we let 36–48 h elapse between detachment and weighing.

Results

The Feeding Model

Because of uncertainty about the trajectory of the decline in host blood energy concentration prior to death, model predictions of lamprey growth were obtained under each of three assumptions (see model description). As expected, under the assumption that host blood energy concentration remained constant at a normal level until death, model predictions of instantaneous growth rates were significantly greater than observed rates (paired t-test, $t = 3.57$, $P = 0.0034$). Also as anticipated, observed growth rates were significantly greater than predicted under the assumption that host blood energy declined precipitously at time Q (equation 4) and remained at a constant low level until host death (paired t-test, $t = 2.43$, $P = 0.030$). Model predictions under the intermediate assumption that host blood energy concentration declined exponentially prior to death were not significantly different from observed values (paired t-test, $t = 0.88$, $P = 0.39$). Under all three assumptions, predicted growth rates were significantly correlated with observed growth rates, but most strongly so for the intermediate assumption ($r = 0.75$, $P < 0.01$; Fig. 5).

Attachment Time Experiment

Mean attachment times for lampreys confined with one and two trout were 15.4 and 10.1d, respectively (Table 1). Although in the direction predicted by the optimal foraging hypothesis, differences between treatments were not significant (paired t-test, $t = 0.84$, $P = 0.42$). Attachment times were highly variable and ranged from less than a day to 70 d. Latency to attack was not significantly different between treatments (paired t-test, $t = 1.24$, $P = 0.24$), although the difference between means was in the direction expected (18.0 and 10.9 d with one and two hosts, respectively; Table 1).

The first attachment, regardless of host density, tended to be longer than the second attachment, with a mean difference of 4.3 d (sign test: $P = 0.022$; signed rank test: $P = 0.048$; paired t-test [omitting case 10 in Table 1]: $t = 3.04$, $P = 0.01$). The same was not true for latency to attack (paired t-test, $t = 0.24$, $P = 0.81$). At each host density, and with both densities combined, attachment time was significantly correlated with the preceding latency to attack ($P < 0.01$), but this was due in all cases to a few extreme data points.

Fifteen of 28 attacks resulted in death of the host (Table 1). In any consideration of lamprey attachment time, it must be

TABLE 1. Results of experiment to test the effect of host density on attachment time of sea lampreys (*Petromyzon marinus*). Individual lampreys (1–14) were exposed to either one trout followed by two trout (order 1–2) or two trout followed by one trout (order 2–1) at 10 ± 0.5°C. Instantaneous growth rates over the duration of two feeding bouts were calculated as [ln(final biomass/initial biomass)]/t, where t is total duration in days of the two feeding bouts, including time spent unattached.

		1 Host Present				2 Hosts Present					
Order of densities	Initial biomass (g)	Host biomass (g)	Latency to attack (days)	Attachment time (days)	Host killed?	Host biomass (g)	Latency to attack (days)	Attachment time (days)	Host killed?	Final biomass (g)	Instantaneous growth rate (per day)
1 1–2	8.53	68.86	12.6	18.3	+	71.63	24.2	9.0	+	9.10	0.0010
2 1–2	6.57	67.00	0.5	24.0	+	54.79	11.0	3.0	+	12.90	0.0178
3 1–2	5.65	60.02	11.1	5.8	−	61.30	2.2	4.5	−	9.32	0.0209
4 1–2	7.35	74.73	13.0	9.1	−	83.89	6.1	7.9	−	13.90[a]	0.0094
5 1–2	7.16	74.45	40.1	37.0	+	80.03	3.0	2.0	+	17.80	0.0111
6 1–2	11.29	58.10	6.0	1.3	+	53.23	0.2	3.5	−	12.66	0.0088
7 1–2	6.62	68.92	23.0	5.0	−	69.23	0.5	3.0	−	7.63	0.0046
8 2–1	7.01	64.20	18.0	9.5	−	66.08	33.1	40.0	+	14.94	0.0074
9 2–1	5.97	49.93	4.5	4.5	−	53.58	5.5	10.5	+	8.05	0.0120
10 2–1	8.78	82.45	62.0	70.0	−	82.35	5.1	1.0	−	19.27	0.0057
11 2–1	10.85	54.93	36.0	1.0	+	54.46	16.2	1.0	−	11.00	0.0003
12 2–1	10.82	94.50	9.5	5.5	−	91.62	2.4	17.0	+	15.96	0.0118
13 2–1	6.38	59.38	14.6	12.9	+	64.88	33.0	22.5	+	11.93	0.0075
14 2–1	5.19	76.00	0.5	11.5	+	69.45	10.5	16.0	+	9.80	0.0167

[a]Not weighed until after an additional feeding.

kept in mind that death of the host may interrupt what might have been a longer attachment. This was probably not important in our comparison of attachment times between the two host densities, as host deaths were about equally split between host densities, and in five cases lampreys killed their hosts at both densities. Nine deaths occurred as a result of first attachments, regardless of host density, while six occurred as a result of second attachments. Lampreys that killed one host were not more likely to kill their second host (G test of independence, $P > 0.25$). Similarly, correlations between attachment times for individual lampreys at the two host densities ($r = -0.26$) and between latencies to attack at the two host densities ($r = -0.067$) were not significant. Thus, variation in feeding behavior was not attributable to consistent differences among individuals.

Growth by lampreys during the experiment was highly variable, at least in part because of great variability in individual feeding histories (Table 1). Increase in wet body weight after two feedings, as a percentage of initial weight, ranged from 1 to 149%.

Discussion

Attachment Time

There are several possible reasons for the lack of significant differences between attachment times for lampreys confined with one and two hosts. Not the least of these is the great variability that seems characteristic of the lamprey feeding process. Nevertheless, this variability was not great enough to prevent the detection of a significant difference between first and second attachments by individual lampreys.

If the previous interval between feedings is used by the lamprey as an approximate measure of current host abundance, then the lack of a significant difference between attachment times at the two host densities may be related to the lack of a significant difference in latency to attack. Thus, the difference in host density did not appear to affect how long it took a lamprey to find and attack a host. We were surprised by the magnitude of the average latency and suspect that it may have been due at least in part to a reluctance to attack hosts as small as those used in this study. Although recently transformed *Petromyzon marinus* have been reported on relatively small fish (e.g., Davis 1967, Mansueti 1962), and we have observed attachments to trout as small as 30 g in our laboratory, most evidence suggests that parasitic lampreys are actively size-selective (Cochran 1985), and we have observed consistently shorter latencies to attack (usually much less than 1 d) for lampreys confined with larger trout. Alternatively, small trout may be more responsive to the physical presence of an approaching lamprey and thus more likely to avoid an attack.

If the energy uptake curve for a feeding lamprey is relatively flat in the neighborhood of the tangent line from the non-feeding time axis, then deviations from the optimal attachment time will not result in greatly reduced rates of energy uptake (Cochran and Kitchell 1986). Thus, according to our feeding model, a 7.4-g lamprey feeding on a 68.2-g trout with a mean latency to attack of 15.62 d (mean values from the first round of our experiment, both host densities combined) could achieve an optimal rate of net energy uptake of 0.34 kJ/d by remaining attached for 11 d (Fig. 6). It could achieve 97% of this rate by remaining attached for 14 d, the predicted host survival time. In light of inevitable variability in such factors as the interval between feedings, lamprey to host biomass ratio, and realized energy intake, differences of this magnitude are not likely to be statistically or biologically significant, and wide variability in attachment times should be expected. Moreover, a general tendency for lampreys to feed beyond the predicted optimal attachment time and kill their hosts (e.g., in the first round of our experiment, mean attachment time was 15 d and 9 hosts were killed) might be expected for two reasons: (1) attachment times greater than the optimum incur smaller penalties in terms of the rate of net energy uptake than attachment times less than the optimum and are less likely to be distinguishable from optimum (Cochran and Kitchell 1986), and (2) the available energy remaining in the host to which a lamprey is currently

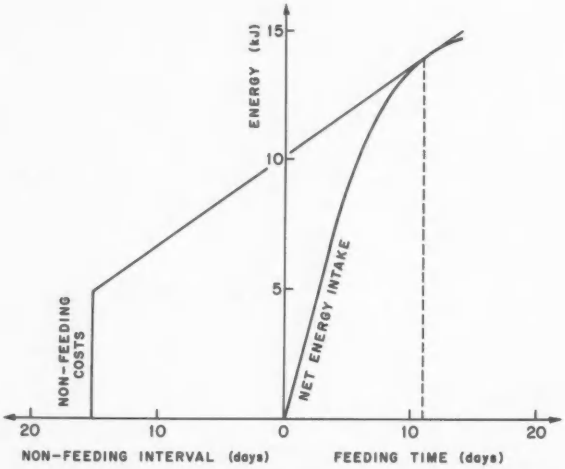

FIG. 6. Predicted net energy intake by a 7.4 g lamprey from a 68.2 g trout as a function of feeding time, with optimal feeding time located at 11 d for a non-feeding interval of 15.2 d. Non-feeding interval and biomass of lamprey and host correspond to mean values from first round of experiment. Energetic costs during the non-feeding interval were calculated with the bioenergetics model of Kitchell and Breck (1980).

attached is, all else equal, a more certain reward than that obtainable by detaching and seeking out a new host (see discussion of risk aversion by Real 1980).

We observed a significant decrease in the attachment time of individual lampreys from the first to the second attachment. Handling times for several sucking insect predators have been observed to decline during sequences of prey captures at constant prey densities (Bailey 1986; Kruse 1983; Giller 1980; and references therein). In one case (Giller 1980), this was associated with declines in the intercatch interval that may have resulted from an increase in search efficiency, but no such decrease in latency to attach was observed in the present study. Decreased attachment time on the seond host may have resulted from growth by lampreys during the experiment. Use of the lamprey feeding and bioenergetics models as outlined above indicated that the experimental lampreys had increased in weight by a mean of 31% by the onset of the second attachment. Because host size was held relatively constant for each lamprey, host blood volume removed daily would increase by a similar percentage (equation 3), and blood quality would decline more quickly during the second attachment (equations 4 and 5). This is corroborated by the fact that in all five cases where lampreys killed both hosts, the second host was killed in a shorter period of time (Table 1).

Alternative Hypotheses

We here consider several possible alternative hypotheses that bear on our assumptions about lamprey foraging behavior. In some cases we are able to provide some insight into the applicability of these hypotheses.

We have assumed that energy is the most appropriate currency by which to evaluate host blood quality. Nutrient constraints may also be important to a consumer (Rapport 1980; Westoby 1978). Youngs and Morris (in Lawrie 1980) suggested that host blood may be a relatively more important source of

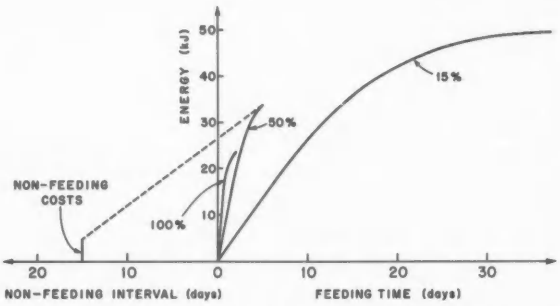

FIG. 7. Predicted net energy intake from a 200-g trout for lampreys remvoing 15, 50, and 100% of the hsot blood volume per day. For a mean non-feeding interval of 15 d, an intermediate removal rate is most profitable in terms of overall rate of net energy intake (slope of dotted tangent line is greater than the tangents to the other curves). See also Cochran and Kitchell (1986).

ions for lampreys in water of low ion concentration. They pointed out that chloride ion concentration is high in the Finger Lakes relative to that in Lake Superior, where lamprey-induced mortality per attack is generally much higher. They also suggested the possibility that ion levels may condition the survival of the host.

The first theoretical considerations of partial prey consumption (Cook and Cockrell 1978; Sih 1980) included the assumption that handling one prey and searching for the next are mutually exclusive activities. However, at least some predators violate this assumption (e.g. Bailey 1985), and predicted feeding times depend on whether or not it is applicable (Lucas and Grafen 1985). The ability to combine search and feeding would allow a lamprey to benefit from the movement of its host by increasing its rate of encounter with new hosts relative to that of a stationary unattached lamprey; several models of predator-prey encounter rates predict that encounter rates are positively related to the rate of predator movement (De Vita et al. 1982; Gerritsen and Strickler 1977; Gerritsen 1980). However, after several months of monitoring lampreys at 12-h intervals under a variety of conditions, we have no evidence that lampreys ever switch directly from one fish to another. With one exception, lampreys were found attached to tank walls or bottoms between consecutive attachments to hosts, and thus it is unlikely that search is combined with feeding.

Unlike the body fluids of prey in previously considered cases of partial prey consumption (Cook and Cockrell 1978; Sih 1980), the blood of a lamprey's host is, to at least some extent, a renewable resource, and Cochran and Kitchell (1986) showed that a lamprey in theory could adjust its rate of blood removal to extend the survival of its host and thereby achieve a greater cumulative uptake of blood. Indeed, one lamprey in our attachment time experiment followed a 62-d period without feeding with an attachment that lasted 70 d but did not kill its host (case 10 in Table 1). During this time it more than doubled in weight. Simulations with the lamprey feeding model suggest that such a strategy should be advantageous when the mean interval between feedings is great (Fig. 7). For the case depicted in Fig. 7, higher long-term rates of net energy intake can be achieved at intermediate rates of host blood removal. Optimization of the rate of net energy intake may necessitate simultaneous consideration of attachment time and rate of host blood removal. This may prove particularly useful in contrasting the feeding behav-

ior of lamprey species from habitats that differ in host abundance or availability.

In considering the problem of how long a lamprey should remain attached to its host, we have considered the individual lamprey as the potential optimizer of time investment. The strategy followed by an individual, however, may depend on the strategies adopted by conspecifics exploiting the same resources. Yamamura and Tsuji (1987) treated the problem of optimal patch residence time under exploitative competition and used a model of resource accrual closely similar to that in Fig. 1. Although some of their particular assumptions about the dynamics of resource supply and exploitation are not applicable to lamprey/host interactions, their approach, based on principles of game theory, may prove especially useful, particularly for lampreys in freshwater systems for which intraspecific competiton may be significant.

The Utility of the Feeding Model

We are encouraged by the performance of the lamprey feeding model in tandem with the bioenergetics model of Kitchell and Breck (1980) in predicting growth by individual lampreys. Figure 5 suggests that these models should provide unbiased estimates of mean feeding and growth rates. Rice and Cochran (1984) enjoyed comparable success with a similar bioenergetics model for largemouth bass (*Micropterus salmoides*).

The feeding model provides a framework for examining a variety of behavioral phenomena. For example, one of the most consistent aspects of lamprey feeding is selective attachment to larger hosts (Cochran 1985; Henderson 1986; Noltie 1987). The feeding model can be used to assess the energetic costs and benefits of size-selective attack and to identify gaps in our understanding of these variables. The significance of host body size to lamprey feeding behavior can be predicted from its effect on the rate of net energy intake over an entire feeding bout, which in turn depends on the effects of host body size on (*a*) the initial slope of the energy intake curve (Fig. 1), (*b*) the peak value of the energy intake curve, and/or (*c*) the duration or energetic cost of the interval between feedings. All else equal, an increase in (*a*) or (*b*) or a decrease in (*c*) will result in an increase in the maximum possible overall rate of net energy intake; potential hosts should be ranked according to this rate.

It is unknown if initial blood quality (energy concentration) varies with host body size, but any such variation would be reflected in the initial slope of the energy intake curve (instantaneous rate of energy intake). In the absence of evidence to the contrary, we have assumed to this point that initial blood quality is a constant, but that assumption should be examined.

Preliminary results suggest that lampreys take longer to penetrate the scale and skin layer of larger hosts (Cochran, unpublished data); Webb and Skadsen (1979) reported that skin density (g/cm^2) increased with body size within several fish species, including rainbow trout. Greater handling times prior to feeding, by increasing factor (*c*) above, would represent an energetic liability of size-selective attack, but handling times are probably in many cases a small fraction of the total interval between feedings.

Two benefits for selecting larger hosts have been proposed (they are not mutually exclusive). First, it has been suggested that greater instantaneous rates of energy intake are achieved on larger hosts (Farmer 1974). Graphically, this means that the initial slope of the energy intake curve (factor *a* above) would be greater for a larger host. There is no evidence for this assertion, and it is contrary to equation (1), which implies that the amount of blood removed does not vary with host size. However, it would be easy to test this hypothesis indirectly by comparing growth rates for lampreys of identical initial size allowed to feed for identical periods on hosts of different sizes. Second, the time of death of a host is inversely related to the proportion of its blood removed daily (Farmer et al. 1975; equation 5). Thus, a lamprey feeding at a given rate is assured of a longer period of feeding on a large host, and a greater cumulative energy intake (factor *b* above).

An obvious limitation of the current feeding model is its restriction to sea lampreys feeding on trout. It would be desirable to extend the model to other lampreys and host taxa, as well as to construct separate curves for attacks in various sites on the body surface of the host (Cochran 1986). Although the logistics of measuring blood consumption by lampreys are formidable (Farmer 1974), recent techniques for repeated sampling of blood from individual cannulated fish (Hoffmann and Lommel 1984; Nichols and Weisbart 1984) may prove applicable to the problem. Also, it may be useful to consider the possible feedback effect of a lamprey's growth on its ability to extract blood from its host, especially during long attachments. No such feedback is incorporated into the current model.

Our experiment on the effect of host density on lamprey attachment times was inconclusive, and the question of whether attachment time is the key to understanding the impact of lampreys in the field remains unresolved. Nevertheless, our lamprey feeding model proved useful in suggesting why it may be hard to demonstrate a significant difference in attachment times (see also Cochran and Kitchell 1986); more importantly, it should prove useful in the efficient design of future experiments. In addition, the model can be used to generate predictions about such feeding behaviors as size selectivity, from either within or outside the context of optimality theory.

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Size-Biased Survival in Steelhead Trout (*Oncorhynchus mykiss*): Back-Calculated Lengths from Adults' Scales Compared to Migrating Smolts at the Keogh River, British Columbia

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Ward, B. R., P. A. Slaney, A. R. Facchin, and R. W. Land. 1989. Size-biased survival in steelhead trout (*Oncorhynchus mykiss*): back-calculated lengths from adults' scales compared to migrating smolts at the Keogh River, British Columbia. Can. J. Fish. Aquat. Sci. 46: 1853–1858.

Lengths of wild, winter-run steelhead smolts, estimated by back-calculation procedures from adults' scales, were compared with observed lengths of migrating smolts sampled near the mouth of the Keogh River, Vancouver Island. Size-biased smolt-to-adult survival rates were estimated for several length categories by utilizing length frequencies from observed smolts, smolt length frequencies which were back calculated from adults' scales, smolt yield, and adult returns. Back-calculated smolt length (BSL) of adults returning from smolts of 1977 to 1982 averaged 193 mm compared to 176 mm for observed smolt length (OSL). Mean BSL was larger than OSL in every year. Adults from odd-numbered smolt years had larger BSL than adults from even-numbered smolt years similar to the pattern in OSL. BSL increased with increased years spent in salt water, based on ageing adults' scales. Males and females had different BSL based on number of years spent in fresh water and salt water, although they exhibited the same mean BSL overall. The smolt-to-adult survival estimates were in close agreement with previous estimates derived from the mean OSL. Predictability of survival in the ocean based on the length of smolts was extended over a broad range of the length distribution of wild steelhead smolts.

Les longueurs de smolts de truite arc-en-ciel en descente hivernale estimées par rétrocalcul à partir d'écailles d'adultes ont été comparées aux longueurs mesurées de smolts en migration recueillis près de l'embouchure de la rivière Keogh sur l'île Vancouver. Nous avons calculé les taux de survie en fonction de la taille, du stade de smolt au stade adulte, pour plusieurs classes de longueur à partir de la fréquence des différentes longueurs de smolts échantillonnés, de la fréquence des longueurs obtenues par rétrocalcul à partir d'écailles d'adultes, du rendement de la production de smolts et de la quantité d'adultes au moment de la montaison. Les longueurs des smolts obtenues par rétrocalcul ("back-calculated smolt length", ou BSL) à partir des écailles d'adultes en montaison qui étaient au stade de smolt de 1977 à 1982 étaient en moyenne de 193 mm, comparé à une longueur moyenne mesurée ("observed smolt length", ou OSL) de 176 mm. La BSL était supérieure à l'OSL à chaque année. Les adultes qui étaient smolts durant les années impaires ont donné des BSL supérieures à celles des adultes qui étaient smolts durant les années paires. Les résultats sont similaires dans le cas des OSL. Nous avons trouvé, en nous basant sur les renseignements fournis par les écailles des adultes, que la valeur des BSL varie en raison directe du nombre d'années passées en eau douce et en raison inverse du nombre d'années passées en eau salée. Suivant le nombre d'années passées en eau douce et en eau salée, les mâles et les femelles ont des BSL différentes, mais, globalement, leurs BSL moyennes étaient identiques. Les estimations du taux de survie du stade de smolt au stade adulte correspondaient étroitement avec les estimations réalisées antérieurement à partir des OSL moyennes. Les prévisions relatives à la survie dans l'océan, prévisions fondées sur la longueur des smolts, ont été étendues pour couvrir un large spectre de la distribution suivant la longueur des smolts de truite arc-en-ciel sauvage.

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Recently, Carlander (1986) summarized the use of fish scales in age and growth studies, and it was evident that there have been few attempts to use scale back-calculation procedures (Ricker 1975) to estimate survival rates of fish. Maher and Larkin (1954) estimated smolt length of steelhead *Oncorhynchus mykiss* (formerly *Salmo gairdneri*) from the Chilliwack River, B. C., using scale samples collected from anglers' catch for the back-calculation exercise, but they did not compare their results with wild smolt data. West and Larkin (1987) used otolith–body length relations and back-calculation procedures to demonstrate that mortality of juvenile sockeye (*Oncorhynchus nerka*) salmon in Babine Lake was size-selective. However, no attempt has been made to employ back-calculation procedures to estimate the smolt-to-adult survival of salmonids as far as we are aware from the scientific literature, nor have scales been used with back-calculation procedures to estimate survivals during the various life stages of wild steelhead.

In an earlier paper, we demonstrated that smolt-to-adult survival for steelhead was positively correlated with smolt size (Ward and Slaney 1988). Our understanding of the relationships between smolt size and smolt-to-adult survival was based on mean smolt size and was thus limited to the range in mean smolt length (160–187 mm). Since a large proportion of migrants

occur outside this range, it was desireable for predictive purposes to expand the range of the steelhead smolt length and survival studies to encompass all smolt sizes evident in the annual seaward migration. In addition, the relationship between smolt size and age-at-return in the previous paper was confounded by smolt age and size interactions since it was not possible to separate the influence of freshwater age from smolt size in that study. We assumed that because fish ages in freshwater years and ocean years were inversely related, and smolt length increased with the number of years spent in fresh water, smolt size at migration was also inversely related to the number of years spent in the ocean. We also suggested that the back-calculated smolt length of males and females be compared from scales of 2- and 3-yr-ocean fish, to provide further evidence of an inverse relationship between smolt length and return age.

Accordingly, the objectives of the current study were: (1) to estimate the size-biased smolt-to-adult survival of steelhead smolts over their range in length, and, (2) to compare observed smolt lengths (OSL) to backcalculated smolt length (BSL) from adult scales of male and female steelhead which had spent 2–4 yr in fresh water, and 1–3 yr in the ocean. We thus required knowledge of the smolt yield of the Keogh River as well as the OSL frequency distributions, the number of returning adults, the age of the returning adults, and the frequency distributions of the BSL, determined from adult scales as the time of ocean entry. The composition of smolts in length intervals was calculated from the length-frequency distribution of smolts and the numerical smolt yield. That composition was compared to the composition of adults in length intervals, which was based on the BSL frequency distribution and adult returns. From these two sources we estimated size-biased survival, utilizing data from 6 yr of smolt yield (1977–82) and subsequent adult returns (1978–85).

Methods

The Keogh River, on northern Vancouver Island, has been described elsewhere (Perrin et al. 1987; Johnston et al. 1986; Slaney et al. 1986; Slaney and Harrower 1981; Ward and Slaney 1981; Smith and Slaney 1980), as have most of the sampling details concerned with this investigation (Ward and Slaney 1988). A fish enumeration fence, 300 m upstream from the mouth of the 33 km river, has been utilized to capture steelhead trout adults, kelts and smolts since 1976.

Calculation of survival rates for the marine phase of steelhead life history required enumeration of both smolts and adults. The number of the adults returning each year was estimated using a Peterson mark–recapture method (Ricker 1975). Detailed methods are provided in Ward and Slaney (1988). Briefly, adults passing upstream through the fish fence had a 6 mm hole punched in their operculum, and kelts passing downstream were examined for this mark. On average, 62% of the population was enumerated as upstream migrant adults or unmarked downstream migrant kelts, 39% of the population was marked while passing upstream, and 23% of the marked males were recaptured while 43% of the marked females were recaptured (Ward and Slaney 1988). Steelhead smolts were enumerated from early April to mid-June at the counting fence using the method described in Ward and Slaney (1988). Briefly, smolts were removed from collection boxes and counted daily, and samples of lengths and scales randomly obtained. Trap efficiency of capture was about 90%, which was determined by marking and releasing large coho salmon smolts (>120 mm) 50 m above the fence (Irvine and Ward 1989).

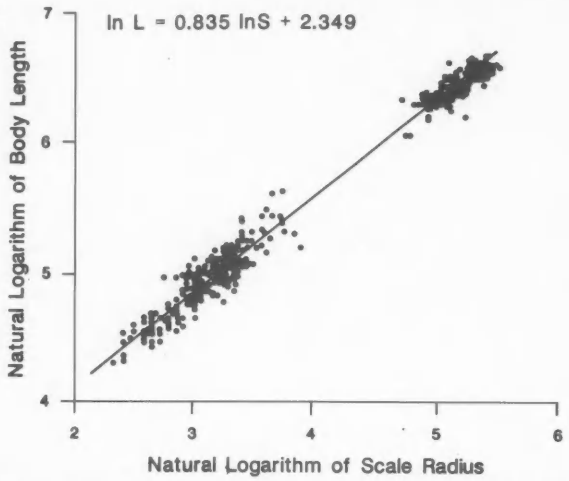

FIG. 1. The relationship between the natural logarithm of fork length (1n L) and the natural logarithm of scale radius (1n S) for steelhead trout of the Keogh River.

Adults were identified by sex, measured for length, and a scale sample was removed from the preferred zone for ageing (Maher and Larkin 1954). Plastic impressions of scales were prepared for analysis (Hooton et al. 1987) and then interpreted with the aid of photomicrographs. The number of years fish spent in the ocean was validated by comparison with marked hatchery fish (Slaney and Harrower 1981). Interpretation of the number of years spent in fresh water was aided by sampling of steelhead parr throughout the river, which assisted us in recognizing annuli formed during their freshwater phase. Estimates of the returns were tabulated based on the year fish were smolts (i.e., the smolt year) and were calculated from the adult population estimates and the age composition of the adult population. Thus, returns after 1, 2, and 3 yr in salt water comprised the total survivors from a smolt year. Fish in their second or greater spawning migration, which on average comprised 10% of the population (Ward and Slaney 1988), were excluded from the analysis.

The back calculation of fish size from scales was dependent on the relationship between fish scale radii and fish length. We obtained a large sample of fish of several life stages from several years, without weighting. Parr, smolt, and adult samples of scales and lengths were obtained from 1977 to 1982 (n = 345) to define this relationship. The Fraser–Lee method of back calculation was used (Ricker 1975; Bartlett et al. 1984; Hooton et al. 1987). Fish > 45 mm only were used in this relationship since many of the fish sampled below this size were within the period of formation of the initial scale placode.

Smolt length was back calculated from 896 adult scales, representing returns from smolts which migrated from the Keogh River between 1977 and 1982. Actual number of adults sampled was higher but some scales (about 20%) were not useful for back calculation due to regeneration in the section of the scale representing freshwater residence. Recognition of the transfer from fresh water to salt water was consistently apparent in adult scales, as described by Maher and Larkin (1954). Reabsorption of the edge (ocean residence) of the adults' scales was not apparent in fish captured moving upstream at the river mouth although this was apparent in kelts migrating to sea. Mean BSL

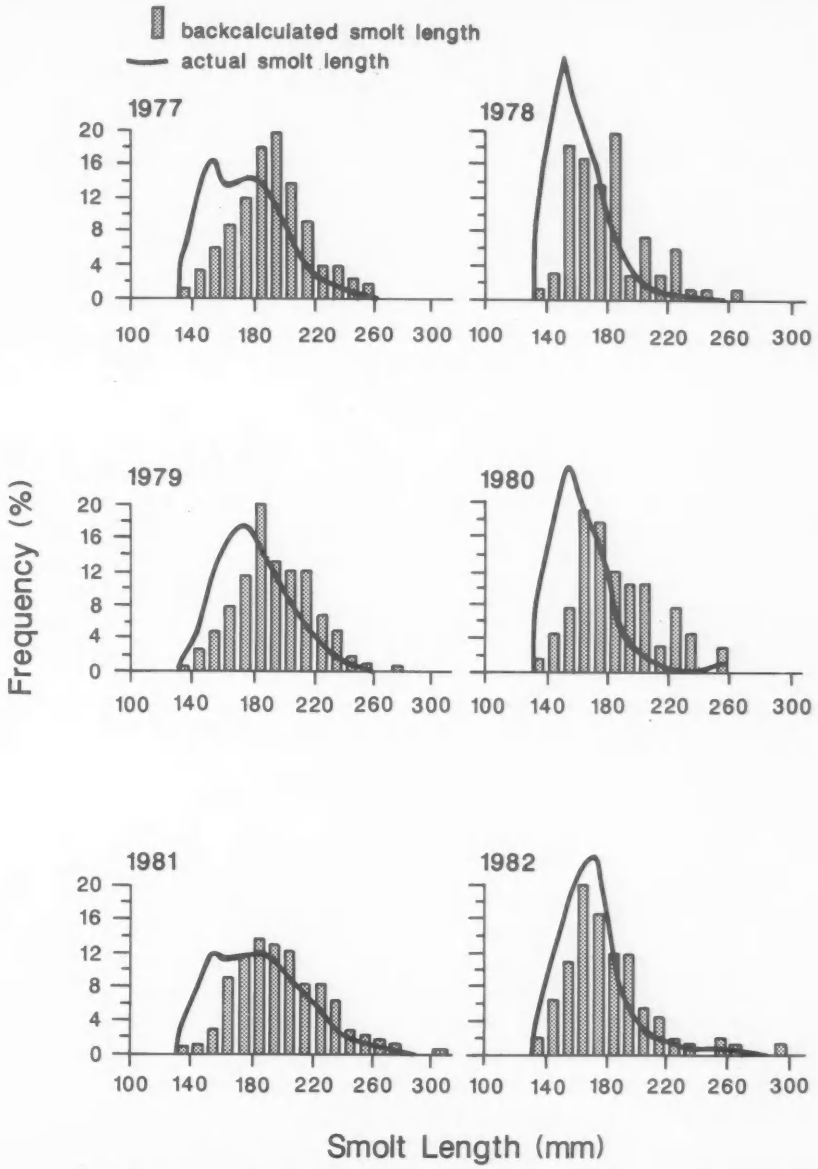

FIG. 2. Frequency distributions of observed (OSL) and back-calculated smolt lengths (BSL) from steelhead trout of the Keogh River for smolt years 1977–82.

from adult fish that were smolts in a given year was compared to the mean size of smolt migrants from that year. With data for all years combined (unweighted), the mean BSL of adults that spent 2 yr in fresh water (age 2.) before becoming smolts (n = 256), 3 yr in fresh water (age 3., n = 561), and 4 yr in fresh water (age 4., n = 77) were calculated and compared to the mean OSL reported for smolts of the same ages in Ward and Slaney (1988). We compared the BSL of males (n = 371) and females (n = 524), and of males and females from different ocean ages (sample size for .1 = 13, for .2 = 623, for .3 = 259).

Estimates of survival of smolts from 1977 to 1982 in six different length groups were based on the mean BSL of each length group. The numerical composition of smolts in their OSL frequency distributions was determined by multiplying the proportion in each length interval by the total number of smolt migrants each year. Similarly, the composition of adult returns within each BSL interval was determined by multiplying the proportion/interval by the number of survivors. The latter was documented by Ward and Slaney (1988) and was estimated by ageing adults to determine composition by age based on years spent in the ocean, and the mark–recapture population esti-

TABLE 1. Observed (OSL) (from Ward and Slaney 1988) and back-calculated lengths (BSL) of Keogh River steelhead smolts in relation to the number of years in freshwater, the number of years in saltwater, the sex of the adult fish, and the year of smolting from the 1977 to 1982 smolt years. SD = standard deviation, n = sample size.

	Mean OSL (mm)	Mean BSL (mm)	BSL SD	BSL n
Years in fresh water				
2	153	177	22.8	256
3	177	196	25.6	561
4	218	220	26.5	77
Years in salt water				
1 males		218	41.5	13
females		—	—	—
2 males		192	25.4	265
females		193	28.5	358
3 males		195	29.5	93
females		188	25.5	166
Sex of adults				
males		192	27.7	524
females		194	27.5	371
Smolt year				
1977	174	193	23.7	185
1978	160	181	26.0	65
1979	180	193	24.2	165
1980	161	186	26.8	68
1981	187	201	28.9	303
1982	170	178	27.8	110

mates. For example, the adult returns from 1977 smolts were comprised of a proportion of the 1978 adults, the 1979 adults and the 1980 adults. Estimates of the number of smolts that migrated within a given size range and of the number of adults they produced permitted calculations of smolt-to-adult survival values for each of the six smolt length intervals. It was necessary to lump data for some intervals, particularly in the larger size categories in some years, to provide adequate sample sizes (>10 estimated survivors). Weighted least squares regression was performed on the survival and smolt length data with all smolt years combined.

Results

A linear relationship was found between the natural logarithms of fork length and scale radius (r^2 = .98, Fig. 1). The relationship was described by the line

$$\ln L = 0.835 \ln S + 2.349$$

where

L = fork length (mm)
S = total scale radius (mm)

Residuals were stabilized and normally distributed after logarithmic transformations.

Because the logarithm of fish length was proportional to the log of the scale radius, with a correction factor (the intercept), we used the modified form of the Fraser–Lee equation for the back calculation of length at age (Bartlett et al. 1984; Hooton et al. 1987):

$$\ln L_a = (\ln L - c) \ln S_a / \ln S) + c$$

where

c = intercept from the regression of ln (fork length) and ln (scale radius)
S_a = scale radius at a given annulus
L_a = fork length of fish at a given annulus

For all smolt years (1977 to 1982), the mean BSL was 192.5 mm, whereas the overall mean OSL was 16.3 less at 176.2 mm (P <0.05). BSL was unimodally distributed and skewed to the right up to 300 mm (Fig. 2). The trend for BSL to be greater than OSL was apparent in every year (Fig. 2).

The mean differences of BSL and OSL ranged from a low of 9 mm in 1982 to a high of 24.5 mm in 1980 (Table 1). Despite this range, the pattern of the mean length over the 6 yr was similar for both BSL and OSL. BSL was normally distributed whereas OSL frequencies were truncated at 130 mm (Fig. 2). Fish smaller than 130 mm were purposely excluded from OSL and considered to be parr, although a few appeared silver. Differences between the shape of the distributions appeared mainly in the lower length intervals. An exception was in 1982, where the BSL frequency distribution was similar to the OSL frequency distribution. Evidently there was relatively less mortality among small smolts in 1982 (Fig. 2).

Mean BSLs were significantly different (P <0.05) among smolt years 1977 to 1982 (Table 1). Adults that had been smolts in 1977, 1979, and 1981 had been consistently larger as smolts than adults that had been smolts in 1978, 1980, and 1982. Similarly, OSLs in the even-numbered years were also smaller (P <0.05; Scheffe's multiple range test) than those in the odd-numbered years.

BSL increased with older age in freshwater years (P <0.05, Table 1), similar to OSL. The BSL of adults that had spent 4 yr in fresh water was >40 mm longer than that of adults that as smolts had spent 2 yr in fresh water. The difference between BSL and OSL of Keogh smolts decreased with increasing number of years spent in fresh water. Adults that had spent 2, 3,

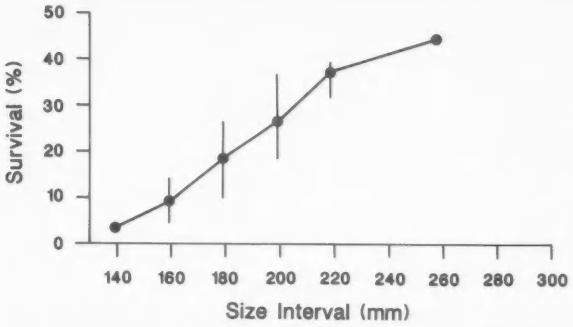

FIG. 3. The weighted least squares regression of steelhead smolt length and smolt-to-adult survival based on the back-calculation of smolt length from adults' scales, for the 1977 to 1982 smolts and subsequent returns. The data points are for all years combined, weighted by sample size; the vertical bars represent the range in survival estimates where sample/interval exceeded 10 fish.

and 4 yr in fresh water had, on average, a BSL that was 24, 16, and 2 mm longer, respectively, than OSL at age. As younger smolts were smaller (Table 1), greatest differences between BSL and OSL were in the smaller size classes, which is consistent with a positive size-survival relationship.

As years spent in the salt water increased, BSL decreased ($P < 0.05$, Table 1) although the difference between age .2 and .3 fish was insignificant when both sexes were considered together. There were differences in the BSL of males and females based on years spent in salt water. Jacks (age .1) were derived from large male smolts but females age .1 were not found. Age .2 males and females had similar BSL, and age .3 females were slightly smaller smolts than males. Males had a significantly larger BSL if they were age .1 rather than ages .2 and .3 ($P < 0.05$), but there was no difference between BSL from .2 and .3 males. However, females aged .3 exhibited significantly smaller (by 5.6 mm) BSL than females aged .2 ($P < 0.05$).

The weighted least squares regression of smolt length and survival was non-significant ($r^2 = .29$, $P > 0.05$) when all smolt years were included in the analysis ($n = 820$ adult scales). With the exclusion of the 1982 smolts (cf Ward and Slaney 1988), the r^2 improved to .74, and the relationship was positive ($P < 0.05$) but non-linear (residuals were not normally distributed). The slope of the line appeared to decrease at both ends of the line similar to a logistic curve, particularly at the largest length interval of 200–260 mm (Fig. 3). Differences in survival appeared large: smolts of 200 mm in length had >8 times the survival of smolts 140 mm in length. The average-sized smolt (176 mm) was predicted to survive at about 17% from the weighted least squares regression equation.

Discussion

The results presented here on smolt length, survival and age-at-return are in agreement with relationships between mean smolt length and survival and age-at-return in Ward and Slaney (1988). The smolt-to-adult survival of steelhead trout was positively related to smolt length, but the range in smolt length examined was greatly extended through the use of back calculation of smolt length from adult scales. At the limits of the curve established by Ward and Slaney (1988) we found similar survivals (differences of less than 2% at 160 and 187 mm) based on the back-calculation method, and the slope and intercepts did not differ. However, we have not identified the mechanisms that result in poorer survival of smaller smolts. Perhaps larger smolts with their greater swimming speed have advantages in both capture of prey (hence more rapid growth than their smaller cohorts) and escape from predators (cf. Taylor and MacPhail 1985). However, the shape of the relationship between survival and smolt length suggests an asymptote is reached at about 250 mm.

There were no differences in the BSL of males and females. Ward and Slaney (1988) did not detect a difference in the sex ratio of male and female smolts within and between 4 size intervals, but their sample size was small. Keogh River steelhead smolts apparently do not display the size-related sex ratio differences found in smolts of some Atlantic salmon populations (Ritter et al. 1986). For modelling of steelhead adult returns based on smolt length, sex ratio differences between length intervals may not be a significant factor. This also implies that there are few, if any, mature male parr in steelhead populations at this latitude.

On average, BSL was lowest for fish that spent more years in salt water. Since years spent in fresh water and years spent in salt water were inversely related (Ward and Slaney 1988) and BSL also was inversely related to years in salt water, it appears larger smolts are the first to mature. However, the maturation rate differs for males and females. Ritter et al. (1986) and Chadwick et al. (1986) provided evidence that there was a parental influence on smolt age and size in Atlantic salmon. If this also applies to steelhead, then adults of older age-at-maturity (based mainly on ocean years) can be expected to yield smaller smolts. Larger adults tend towards larger egg size and subsequent fry size (Gall 1974) and fingerling size (Fowler 1972) which, for steelhead, may result in faster growth in general and earlier smoltification. However, this may also be further affected by the rearing environment; for example, a fish rearing in the cold headwaters or in relatively cold years may smolt at a later age (and possibly a larger smolt size) than under other conditions, despite the parental influence. This may explain why Hooton et al. (1987) found no correlation between adult size and BSL of angler-caught steelhead from Vancouver Island — the highly variable coastal stream environment and plasticity in life history traits could mask any genetic effect. Several other studies of salmonid life history have demonstrated a relationship between smolt size and age-at-return (Hyatt and Stockner 1985).

Hyatt and Stockner (1985) also noted that the timing of the smolt migration may play a significant role in both the age-at-return and survival in the ocean. It was not possible for us to separate smolt timing effects in the back-calculation exercise. However, we noted that smolts occurring early in the smolt migration were consistently larger (by an average of 10 mm, $P < 0.05$, data on file) than smolts occurring at the peak migration time, a trend also observed with Keogh coho smolts (Irvine and Ward 1989). To provide a more definitive examination, the interactions of size with timing of smolt migrations should be examined in wild steelhead, along with the relationship between survival and age-at-return. We are unsure if the differences in smolt size between odd- and even-numbered smolt years is related to smolt-timing effects or a function of other ecological relationships during the freshwater rearing stage (e.g., variation in odd- and even-year pink salmon (*Oncorhynchus gorbuscha*) abundance — Johnston et al. 1986).

A variety of back-calculation techniques to estimate smolt size of steelhead trout have been reported (Maher and Larkin 1954; Chapman 1958; Narver and Withler 1971; Narver and

Anderson 1974; Whately 1977; Horncastle 1981). Hooton et al. (1987) established a back-calculation procedure mathematically similar to Bartlett et al. (1984) yet separately and almost simultaneously derived. However, Hooton et al. (1987) dealt almost entirely with angler-caught fish, sample sizes were low in many cases, and there was no test of the back-calculation model against empirical data. The similarity between survival rates based on mean smolt length (Ward and Slaney 1988) and rates based on BSL provided evidence that the back-calculation method of this paper was valid.

Variation in the difference between BSL and OSL further suggested that conditions for survival in the ocean can change. This was particularly evident for the 1982 smolts which experienced higher survival of the smaller size classes compared to other years. Changes associated with the 1982–83 El Niño event may possibly be correlated with increased survival of steelhead post-smolts at this latitude (Ward and Slaney 1988; Wooster and Fluharty 1985). Despite this variation, the descriptive model of Ward and Slaney (1988) should incorporate the new evidence of this paper to perhaps assist in the forecasting of returns of adult steelhead trout based on smolt length. Given longer term data on climatic change (e.g., sea-surface temperature) and smolt survival, the former might be included with variables in the prediction of adult returns. To gain an understanding of the role of the marine environment in size-biased smolt survival and age-at-return will require greater research into the early sea-life of steelhead trout.

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The Importance of Activity in Bioenergetics Models Applied to Actively Foraging Fishes[1]

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Boisclair, D., and W. C. Leggett, 1989. The importance of activity in bioenergetics models applied to actively foraging fishes. Can. J. Fish. Aquat. Sci. 46: 1859–1867.

We used the Kitchell et al. (J. Fish. Res. Board Can. 34: 1922–1935) bioenergetics model and field derived estimates of growth and consumption rates to estimate the quantity of energy allocated to activity by 28 combinations of yellow perch (*Perca flavescens*) age class and population. Activity costs among populations ranged from 0 to 40% of the perch bioenergetics budget. We further evaluated the influence of activity rates on the food consumption estimates predicted by the Kitchell et al. model and the model proposed by Kerr (Can. J. Fish. Aquat. Sci. 39: 371–379). As suggested by Kerr, activity costs increased as food consumption increased. However, we found no significant relationship between predicted and observed food consumption estimates for either model. The magnitude of, and the among-population variance in, the quantity of energy allocated to activity is consistent with our hypothesis that this component of the bioenergetics budget of fishes has the potential to contribute meaningfully to the explanation of inter-population differences in perch growth and, by extension, to the variance in growth of other actively foraging fish species.

Nous avons utilisé le modèle bioénergétique de Kitchell et al. (J. Fish. Res. Board Can. 34: 1922–1935) et des données de terrain sur les taux de croissance et de nutrition pour estimer les taux d'activité de 28 combinaisons de classes d'âges et de populations de perchaudes (*Perca flavescens*). Les coûts reliés à l'activité ont varié, selon les populations, entre 0% à 40% du budget énergétique des perchaudes. Nous avons aussi évalué l'influence des taux d'activité sur les prédictions des taux de consommation de nourriture des modèles de Kitchell et al. et de Kerr (Can. J. Fish. Aquat. Sci. 39: 371–379). Tel que suggéré par Kerr, les taux d'activité ont augmenté avec les taux de consommation de nourriture. Nous n'avons pas trouvé de relation significative entre les valeurs prédites et observées des taux de consommation de nourriture pour aucun des deux modèles. L'importance et la variabilité inter-population de la quantité d'énergie allouée à l'activité indiquent que cette composante des modèles bioénergétiques peut grandement contribuer à la variabilité inter-population de la croissance des perchaudes, et par extension, celle d'autres espèces de poissons qui s'alimentent de façon active.

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We recently analyzed the combined influence of the quantity of food consumed, diet composition and fish density on fish growth (Boisclair and Leggett 1989a,b,c). These analyses led us to hypothesize that energy costs associated with activity were major contributors to inter-population differences in the growth of yellow perch (*Perca flavescens*) and, by extension, to other planktivorous and/or benthivorous fishes. For this hypothesis to be valid, activity costs must account for a large and variable fraction of the bioenergetics budget of perch.

Direct assessments of the absolute and relative quantities of energy allocated to activity in free living fishes are lacking, presumably because of the logistic difficulties involved. For this reason, the activity component of bioenergetics models is generally assumed and, unlike the situation for most other parameters in these models (i.e. standard metabolic rate, specific dynamic action, urinary and fecal losses), this assumption is usually not well founded on the results of experimental data. Kitchell et al. (1977), in their application of bioenergetics models to the prediction of food consumption in perch, assumed that the ratio ((standard metabolism + activity)/ standard metabolism) was constant. In contrast, Kerr (1982), following a somewhat different approach to bioenergetics modelling, hypothesized that the energy allocated to activity was variable and positively correlated with the quantity of food consumed. In consequence, bioenergetics models, when applied to predict growth or food consumption in fishes, typically contain two unknowns: activity, which is assumed, and one of growth or food consumption.

Our analyses of the biological variables explaining inter-population differences in growth rates (Boisclair and Leggett 1989a,b,c) have provided the data necessary to reduce the number of unknowns to one, thereby facilitating a direct assessment of the magnitude and variance of the energy allocated to activity in free-ranging perch occupying different habitats.

In this paper, we apply these data to the models proposed by Kitchell et al. (1977) and Kerr (1982) in a direct test of their

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TABLE 1. Observed total food consumption (C_0) from May to September and its allocation to different physiological compartments (G_0 = growth observed, SMR_0 = standard metabolism, A_0 = activity) for the 28 combinations of perch age class and population studied (A·P). Population identification number (ID) are presented in parentheses. Estimates of SMR_0 and A_0 were derived from the Kitchell et al. (1977) model by substitution of our field derived growth and food consumption rates into the model. Specific dynamic action (SDA = 15–17% of C_0), nitrogen excretion (E = 10–14% of C_0) and fecal losses (F = 10–15% of C_0) were very stable and are not presented.

A·P	ID	C_0 (g wet)	G_0 (% of C_0)	SMR_0 (% of C_0)	A_0 (% of C_0)	$(A_0 + SMR_0)/SMR_0$
Drolet I	(5)	78.0	22.1	21.3	16.2	1.76
Silver I	(11)	50.5	23.3	24.5	11.7	1.48
Massawippi I	(8)	36.0	14.4	19.3	25.3	2.31
Memphremagog I	(9)	33.5	25.9	27.9	5.4	1.19
D'Argent I	(4)	31.4	26.4	45.8	−12.1	0.74
Brompton I	(3)	28.5	16.2	26.5	16.3	1.61
Bromont I	(2)	17.2	27.9	66.6	−39.4	0.48
Waterloo I	(12)	12.2	49.4	78.6	−67.9	0.14
Silver II	(11)	191.4	16.4	18.4	23.7	2.28
Magog II	(7)	107.9	13.2	18.7	26.6	2.42
Memphremagog II	(9)	80.6	13.7	23.8	21.4	1.90
Brompton II	(3)	78.6	11.0	21.0	26.2	2.25
Massawippi II	(8)	74.2	2.4	16.3	39.7	3.44
D'Argent II	(4)	66.7	3.6	38.0	18.4	1.48
Roxton II	(10)	57.2	10.5	31.8	16.6	1.52
Hertel II	(6)	46.9	5.0	23.7	30.1	2.27
Bromont II	(2)	45.1	9.8	53.7	−4.0	0.93
Waterloo II	(12)	31.0	17.4	63.7	−21.0	0.67
Drolet III	(5)	204.6	6.3	17.5	35.2	3.01
Magog III	(7)	192.2	4.8	16.2	36.8	3.27
Brome III	(1)	172.4	2.3	14.1	40.7	3.88
Brompton III	(3)	132.1	5.0	18.7	34.0	2.82
Memphremagog III	(9)	118.6	8.6	22.4	27.8	2.24
Roxton III	(10)	99.0	7.1	27.9	23.8	1.85
D'Argent III	(4)	97.2	5.8	35.4	18.5	1.52
Bromont III	(2)	76.0	5.6	49.0	4.8	1.10
Hertel III	(6)	70.2	2.8	21.9	33.8	2.54
Waterloo III	(12)	42.3	30.0	60.4	−30.3	0.50

assumptions regarding activity costs. We further assess the influence of these assumptions on the food consumption estimates predicted by the two models.

Methodology

Data Source

We determined the growth, the diet composition, and the quantity of food consumed by yellow perch (*Perca flavescens*) of ages I+, II+, and III+ in each of 12 populations (See Boisclair and Leggett 1989a: fig. 1). These estimates were made during the interval May to September 1985 (Boisclair and Leggett 1989a,b). These data were used as inputs to the bioenergetics models of Kitchell et al. (1977) and Kerr (1982).

Covariance analyses performed on each combination of perch age class and population indicated that sex did not contribute significantly to differences in the mean weight of perch within a given population ($P > 0.05$; Boisclair and Leggett 1989a). This result is consistent with previous findings for perch (Ney and Smith 1975; Nakashima and Leggett 1975; Hansson 1985). We therefore pooled sexes in all analyses and simulations.

The Bioenergetics Models

Kitchell et al. (1977) used a series of studies on fish physiology and bioenergetics (Solomon and Brafield 1972; Kelso 1972; Schneider 1973; Swenson and Smith 1973; Beamish 1974; Neil and Magnuson 1974; Kitchelll et al. 1974; Elliott 1976 a,b; Hokanson 1977) to construct a bioenergetics model for perch. This model has the form;

$$(1)\quad G = C - (SMR + A + SDA + E + F)$$

where "G" is the growth (somatic + gonadal), "C" is the quantity of food consumed, "SMR" is the standard (maintenance) metabolism, "SDA" is the specific dynamic action (energy utilized for the mechanical and biochemical processing of the food ingested), "E" and "F" are respectively the nitrogen excretion (urinary and gill excretion) and the fecal losses and "A" is the energy allocated to activity. In this paper, all terms of equation 1 are expressed in g wet · g wet^{-1} · d^{-1}.

The Kitchell et al. (1977) model is most often used to estimate the quantity of food consumed by fish from growth and water temperature data. Standard metabolic rate (SMR) is estimated from fish weight and water temperature (Fry 1957; Bevelhimer et al. 1985; Kiorboe and Mohlenberg 1987; Lemons and Crawshaw 1987) while specific dynamic action (SDA), nitrogen excretion (E) and fecal losses (F) are estimated from the quantity of food consumed and water temperature (Elliott 1976a, b; Brett and Groves 1979; Jobling 1983; Beamish and MacMahon 1988). The Kitchell et al. (1977) model, and its computer version (Hewett and Johnson 1987), assume that

active metabolism (SMR + A) is a fixed multiple of standard metabolism (SMR). In their appendix 4, Hewett and Johnson (1987) suggest that, for perch, an appropriate multiple is 1 (see also Kitchell et al. 1977). This assumption implies that the energy allocated to activity (A) is close to 0. Both Kitchell et al. (1977) and Hewett and Johnson (1987) estimated the quantity of food consumed by fish by iterative approximations that define the average proportion (P) of the maximum consumption rate providing the best fit to the observed growth under specified model parameters. The functions and parameters employed to derive the terms of equation 1 are detailed in Kitchell et al. (1977) and Hewett and Johnson (1987).

Kerr (1982), using a framework similar to equation 1, and assumptions on the relationship between the maximum quantity of food consumed, maximum metabolism and maximum activity levels, hypothesized that the quantity of energy expended on activity by an actively foraging fish should be positively and linearly related to the quantity of food consumed. Kerr's (1982) model (rearranged) is:

$$(2)\quad A = \frac{(C - SMR/\tau) \cdot A_m'}{(C_m - SMR/\tau)}$$

where "C_m" is the maximum food consumption rate, "A_m" is the upper physiological limit of activity rate when the fish is fed at its maximum food consumption rate, and "τ" is the proportion of the food energy consumed that is assimilated minus the proportion of energy consumed that was used for SDA. The units employed in equation 2 were g wet · g wet^{-1}·d^{-1}.

Estimation of Activity Costs

We applied the Kitchell et al. (1977) model to each of 28 combinations of perch age class and population for which we had data (Boisclair and Leggett 1989a) in order to estimate activity by difference (Table 1). In so doing we replaced their algorithm for estimating food consumption by our field derived values (Boisclair and Leggett 1989a). We used linear interpolation (Kitchell et al. 1977; Hewett and Johnson 1987) to define model values between the four sampling dates.

We also employed equation 2 (Kerr's model) to estimate daily activity costs for each of the 28 combinations of perch age class and population we studied (Table 2). All parameters, other than food consumption, required to solve equation 2, were taken from Kitchell et al. (1977) and Hewett and Johnson (1987). We used our estimates of food consumption (Boisclair and Leggett 1989a). Model values between sampling days were estimated by linear interpolation.

We used a series of Monte Carlo simulations to evaluate the influence of the error around our field derived values of food consumption on the activity estimates determined using the Kitchell et al. (1977) and the Kerr (1982) models. For each of the 28 data sets analyzed we calculated 1000 total activity costs using the Kitchell et al. model and 1000 total activity costs using the Kerr (1982) model. Individual activity values were derived using food consumption estimates drawn in relation to their frequency distributions as bounded by the 99.9% C.I. determined in the field (Boisclair and Leggett 1989a).

Influence of Activity Costs on the Predictions of Food Consumption

To illustrate the influence of the magnitude, and the variability, of activity costs on the output of the Kitchell et al. (1977) model we used the computer version of their model (Hewett and Johnson 1987) to predict the food consumption for the 28 combinations of perch age class and population for which we had data. In these simulations we followed their assumption that (SMR + A)/ SMR = 1. Food consumption was estimated using their iterative procedure.

TABLE 2. Total quantity of energy allocated to activity (A_p) from May to September as predicted from the Kerr (1982) model. See Table 1 for the definition of terms.

A-P	A_p (g wet)	A_p (% of C_0)	$(A_p + SMR_0)/SMR_0$
Drolet I	17.0	21.8	2.02
Silver I	11.7	23.2	1.95
Massawippi I	9.7	26.8	2.39
Memphremagog I	8.4	25.2	1.90
Brompton I	5.8	20.3	1.77
D'Argent I	3.0	9.6	1.21
Bromont I	−0.9	−5.4	0.92
Waterloo I	−1.6	−13.4	0.83
Silver II	60.4	31.6	2.71
Magog II	33.7	31.2	2.67
Massawippi I	28.4	30.1	2.85
Memphremagog II	24.7	30.6	2.28
Brompton II	20.8	26.4	2.26
Roxton II	14.4	25.1	1.79
Hertel II	10.3	21.9	1.92
D'Argent II	10.3	15.5	1.41
Bromont II	2.3	5.2	1.10
Waterloo II	−1.1	−3.7	0.94
Magog III	66.6	34.7	3.14
Brome III	62.0	35.9	3.54
Drolet III	56.3	27.5	2.57
Brompton III	39.9	29.5	2.58
Memphremagog III	38.6	32.6	2.45
Roxton III	29.8	30.1	2.08
D'Argent III	17.8	18.3	1.52
Hertel III	17.0	24.3	2.11
Bromont III	7.4	9.7	1.20
Waterloo III	−0.5	−1.1	0.98

Based on his assumption of a linear relationship between activity and consumption rates, Kerr (1982) suggested that the quantity of food consumed by fish can be estimated as:

$$(3)\quad \tau C = \frac{(\tau C_m - SMR) \cdot G}{pC_m - MMR} + SMR$$

where "P" is the proportion of the food energy consumed that is assimilated and "MMR" is the maximum sustained total metabolism. We used equation 3 to predict food consumption by the 28 combinations of perch age class and population. All parameters, other than growth, required to solve equation 3 were derived from Kitchell et al. (1977) and Hewett and Johnson (1987).

Comparison of the Predictions of Bioenergetics Models

The components of the perch bioenergetics models were calculated independently for each combination of perch age class and population as the sums of the quantity of energy allocated to these components during the sampling interval. These sums were standardized by dividing by the total quantity of energy allocated to standard metabolism during the sampling interval.

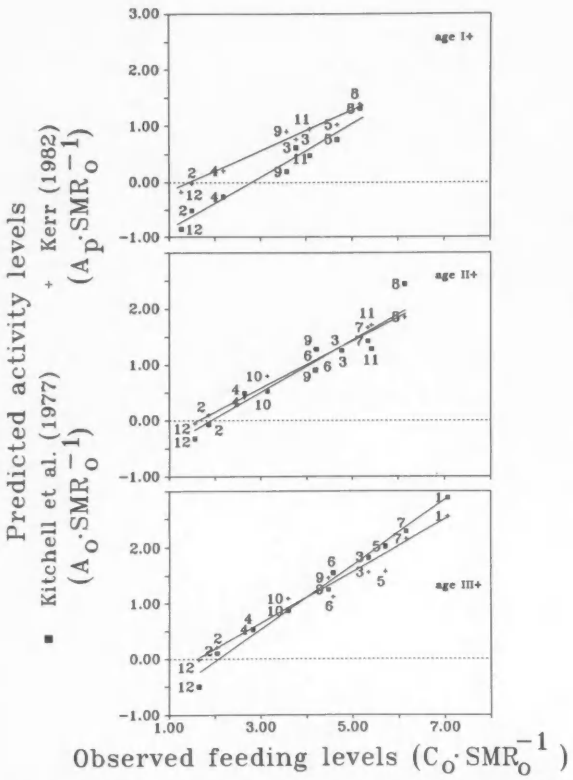

FIG. 1. Relationships between activity costs determined using the Kitchell et al. (1977) or the Kerr (1982) models and observed feeding levels (C_o/SMR). Activity and consumption levels are standardized by dividing by the standard metabolic rate. Populations are identified in Table 1.

This standardization has several advantages: (1) it allows an immediate assessment of the assumption that active metabolism is a fixed multiple of SMR, (2) it permits a direct comparison of our results to those of Kerr (1982) who also standardized his results with SMR, (3) it partially accounts for within perch age class variability in fish weight, and (4) it compensates for among-population differences in sampling interval.

Results

Estimation of Activity Costs

Bioenergetics models could not be balanced for 6 of the 28 combinations of perch age class and population for which we had data. In these cases, which involved fish from populations which typically consumed low quantities of food, substitution of our growth and feeding data into the Kitchell et al. (1977) bioenergetics models yielded negative activity estimates (Table 1).

Maximum total activity costs estimated by difference using the Kitchell et al. (1977) model were equivalent to 12.6 g wet for age I+ (Lake Drolet), 45.3 g wet for age II+ (Lake Silver), and 72.0 g wet for age III+ fish (Lake Drolet). Activity costs represented as much as 25.3% of the total bioenergetics budget of age I+. Corresponding values for age II+ and III+ fish

TABLE 3. a) Statistics of the relationships between predicted activity costs (A_o/SMR; determined by difference using Kitchell et al. (1977) model) and observed feeding levels (C_o/SMR). b) Statistics of the relationships between predicted activity costs A_p/SMR; Kerr (1982) model) and observed feeding levels (C_o/SMR). c) Statistics of the relationships between the predicted activity costs (A_p/SMR; Kerr 1982) and those determined by difference from the Kitchell et al. (1977) model (A_o/SMR; when field values of growth and consumption rates were used as inputs). Degrees of freedom associated with F values are 1,6 for age I+ fish, 1,8 for age II+, and 1,8 for age III+ fish.

	Age class	Model	F	P	r^2
a)	I+	$A_o/SMR = 0.48\ C_o/SMR - 1.38$	144.3	0.0001	0.96
	II+	$A_o/SMR = 0.49\ C_o/SMR - 1.01$	89.1	0.0001	0.92
	III+	$A_o/SMR = 0.58\ C_o/SMR - 1.22$	613.1	0.0001	0.99
b)	I+	$A_p/SMR = 0.38\ C_o/SMR - 0.60$	246.2	0.0001	0.98
	II+	$A_p/SMR = 0.43\ C_o/SMR - 0.68$	272.5	0.0001	0.97
	III+	$A_p/SMR = 0.45\ C_o/SMR - 0.72$	213.2	0.0001	0.96
c)	I+	$A_p/SMR = 0.74\ A_o/SMR + 0.47$	86.2	0.0001	0.93
	II+	$A_p/SMR = 0.77\ A_o/SMR + 0.29$	40.8	0.0002	0.84
	III+	$A_p/SMR = 0.76\ A_o/SMR + 0.24$	137.5	0.0001	0.95

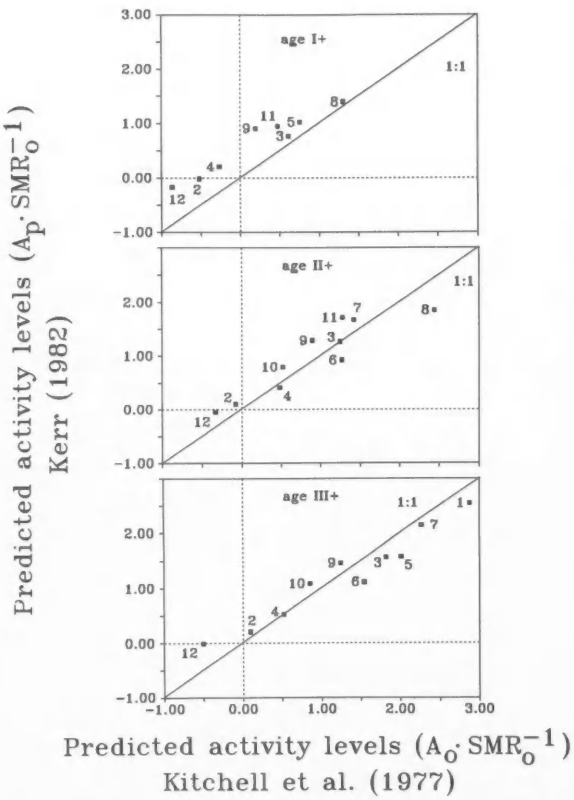

FIG. 2. Relationships between predicted activity costs (A_p/SMR; Kerr 1982) and those determined by difference using the Kitchell et al. (1977) model (A_o/SMR). Activity rates are standardized by dividing by the standard metabolic rate. Populations are identified in Table 1.

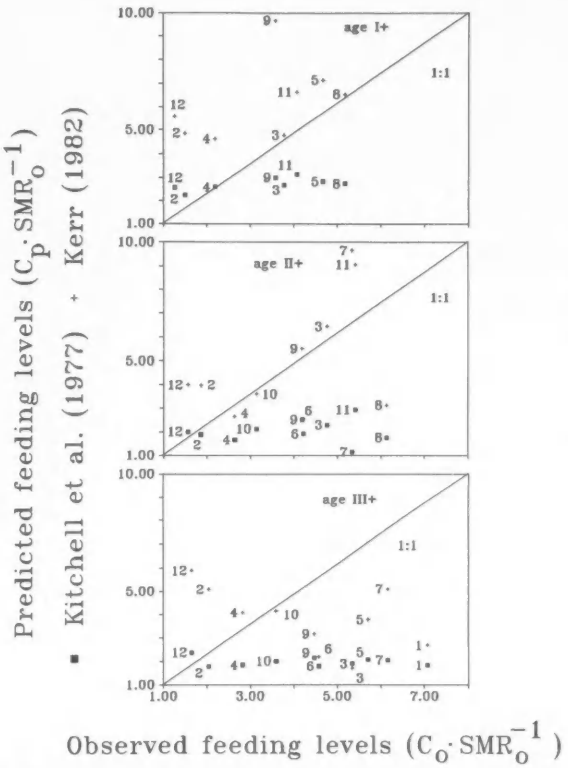

FIG. 3. Relationship between predicted (C_p/SMR) and observed feeding levels (C_o/SMR). Consumption rates are standardized by dividing by the standard metabolic rate. Populations are identified in Table 1.

TABLE 4. Predicted (C_p) total food consumption by perch. Predictions are based on the Kitchell et al. (1977) and the Kerr (1982) models. See Table 1 for the definition of terms.

	C_p (g wet)	
A·P	Kitchell et al. (1977)	Kerr (1982)
Drolet I	67.2	118.8
Silver I	39.2	82.0
Massawippi I	25.7	45.4
Memphremagog I	32.2	90.3
D'Argent I	40.6	66.5
Brompton I	20.7	36.1
Bromont I	26.1	55.7
Waterloo I	44.2	53.5
Silver II	103.0	320.0
Magog II	59.1	117.6
Memphremagog II	45.0	91.6
Brompton II	37.9	106.4
Massawippi II	25.9	37.8
D'Argent II	44.7	66.9
Roxton II	40.2	104.2
Hertel II	34.0	28.3
Bromont II	65.9	95.7
Waterloo II	58.1	78.9
Drolet III	84.7	135.1
Magog III	68.5	176.8
Brome III	46.2	176.1
Brompton III	50.6	70.0
Memphremagog III	53.1	110.2
Roxton III	55.3	138.1
D'Argent	54.8	140.5
Bromont III	48.6	190.0
Hertel III	43.5	33.6
Waterloo III	83.4	151.0

were 39.7% and 40.7% respectively. On average, we found that 3.4 times more energy (g wet) was allocated to activity than to growth (Table 1). The ratio of active metabolism (SMR + A) to standard metabolism (SMR) ranged from 0.14 to 2.31 for age I+, 0.67 to 3.44 for age II+ and from 0.50 to 3.88 for age III+ fish (Table 1).

Total activity costs estimated with the Kerr (1982) model averaged 6.6 g wet for age I+, 20.4 g wet for age II+ and 33.5 g wet for age III+ fish and respectively represented 13.5%, 21.4% and 24.2% of the perch bioenergetics budget (Table 2). The ratio of active metabolism (SMR + A) to standard metabolism (SMR) estimated with the Kerr (1982) model ranged from 1.21 to 2.39 for age I+, from 0.94 to 2.85 for age II+, and from 0.83 to 3.54 for age III+ fish (Table 2).

There was a strong, positive, linear relationship between the activity costs (calculated by difference with both the Kitchell et al. (1977) and the Kerr (1982) models) and observed food consumption for the 3 perch age classes examined (Table 3a, b; Fig. 1).

There was also a highly significant positive correlation between the activity costs calculated for the 3 perch age classes examined using the Kerr (1982) model and those estimated using the Kitchell et al. (1977) model (Table 3c; Fig. 2). Under the model parameters utilized, the Kerr (1982) model generally gave slightly higher estimates of activity costs than did the Kitchell et al. (1977) model. The relationships for age I+ and III+ had slopes significantly different from unity (age I+: $t_6 = 3.25$; $P < 0.05$; age II+ : $t_8 = 1.90$; $P > 0.05$; age III+: $t_8 = 4.00$; $p > 0.01$) and intercepts significantly different from 0 (age I+: $t_6 = 8.25$; $P < 0.001$; age II+ : $t_8 = 1.99$; $P > 0.05$; age III+: $t_8 = 2.33$; $P < 0.05$).

Monte Carlo simulations involving the Kitchell et al. (1977) model indicated that the error associated with our field derived food consumption values (average 95% C.I. = mean ± 35%) induced ± 86.1% errors in our estimates of total activity costs (g wet). The 95% C.I. for the contribution of activity to bioenergetics budget and the (SMR + A)/SMR ratio were ± 65.4% and ± 88.0%, respectively. These analyses indicate that the 6 negative activity costs derived can not be statistically distinguished from 0. This suggests that in some situations activity costs can approach 0. The 95% C.I. around activity costs determined using the Kerr (1982) model were ± 82.0% (total activity), ± 52.6% (percent of total budget allocated to activity) and ± 87.3 (SMR + A/SMR ratio).

Influence of Activity Costs on the Predictions of Food Consumption

The total quantity of food consumed, as predicted with the Kitchell et al (1977) model given the assumption that (SMR + A)/SMR = 1, averaged 37.0 g wet for age I+, 51.4 for age II+ and 58.8 for age III+ fish (Table 4). Correspond-

ing field derived values were 35.9, 77.9, and 120.4 g wet, respectively (Table 1). We found no significant relationship between the food consumption predicted by the Kitchell et al. (1977) model and the field derived values under the assumption (SMR + A)/SMR = 1 (Fig. 3; age I+: $F_{1,6} = 4.98$; $P > 0.06$; age II+: $F_{1,8} = 0.07$; $P > 0.75$; age III+: $F_{1,8} = 0.42$; $P > 0.54$).

The total quantity of food consumed predicted by the Kerr (1982) model averaged 68.5 g wet for age I+, 104.7 for age II+ and 132.1 for age III+ fish (Table 4). We found no significant relationship between the feeding levels predicted using the Kerr (1982) model and those observed (Fig. 3; age I+: $F_{1,6} = 1.63$; $P > 0.24$; age II+: $F_{1,8} = 2.53$; $P > 0.15$; age III+: $F_{1,8} = 3.89$; $P > 0.08$).

Discussion

Magnitude and Variability of Activity Costs

Our analyses indicate that, for perch, activity costs can account for a large fraction (up to 40%) of the bioenergetics budget. Moreover, activity costs can vary widely among populations. These results indicate that the use of a constant multiple of the standard metabolic rate as a measure of activity costs in bioenergetics models of actively foraging fishes (and/or errors in the determination of these activity costs) will induce large errors in the growth and food consumption values predicted by these models (Kelso 1972; Healey 1972; Minton and McLean 1982; Diana 1983; Kerr and Dickie 1985). These results emphasize the importance of viewing activity as a truly independent variable in bioenergetics models.

Influence of Activity Costs on the Predictions of Food Consumption

The most interesting feature of the Kitchell et al. (1977) model is its flexibility. The model can accommodate both changes in the ratio of active to standard metabolism (SMR + A/SMR), and adjustments of the "P" value (fraction of maximum consumption actually realized) during arbitrarily defined intervals. Rice et al. (1983) and Rice and Cochran (1984) demonstrated that the use of different "P" values during distinct seasonal intervals significantly improved the accuracy of predicted growth estimates for largemouth bass (*Micropeterus salmoides*). These authors concluded that the variability in "P" reflected seasonal variability in prey availability. From a modelling perspective, increases in the value of "P" are similar to decreases in the ratio of active to standard metabolism. However, the ecological significance of these two changes is dramatically different in the case of actively foraging fish such as perch. Increases in "P" imply an increase in food consumption rates. Decreases in the ratio of active to standard metabolism imply a decrease in activity rates, possibly in response to increases in prey availability. In this sense when used to estimate the quantity of food consumed by actively foraging fish, the Kitchell et al. (1977) model, and its computer version (Hewett and Johnson 1987) represent open-ended equations. A single data set of fish weight (and fish caloric density), water temperature, and fish diet can give rise to a multitude of sets of consumption estimates simply by varying the "P" value via changes in the assumed ratio of active and standard metabolism. Any reasonable alteration of the assumption regarding the SMR + A/SMR ratio (i.e. range of 1 to 4) is compensated by a change in the estimate of the quantity of food consumed without compromising the fit to the observed growth trajectory.

To illustrate this point, in our study, a change in the (SMR + A)/SMR ratio from 1 to 2.31 for age I+ fish from Lake Massawippi resulted in a 40% increase in the estimate of total consumption. Similarly, a (SMR + A)/SMR ratio of 1 rather than the value of 3.88 observed for age III+ fish from Lake Brome reduced total consumption to 46.2 g wet from the observed value of 172.4; 3.7 fold difference. While we acknowledge that the Kitchell et al. (1977) model would give the observed food consumption estimate if initialized with the correct (SMR + A)/SMR ratio, there is no way to assess the appropriate ratio, a priori, and, therefore, no way to determine which of the numerous series of food consumption and activity estimates obtained by varying this ratio correspond to in situ realities. Consequently, the production of a well balanced bioenergetics model that provides a good fit to the growth curve of an actively foraging fish does not validate the food consumption estimates predicted by this model. We conclude that the Kitchell et al. (1977) model and the Hewett and Johnson (1987) computer version of this model cannot be trusted to reliably predict food consumption for actively foraging fish.

The Kitchell et al. (1977) model becomes a valuable tool in the study of fish bioenergetics only when employed with one unknown. This requirement can be met under two circumstances: First, when reliable field-derived growth and food consumption rates are available, thereby allowing assessment of activity costs. Second, when independent data sources indicate that activity costs are, indeed, negligible. This second condition appears to apply to piscivorous, or sit-and-wait, ambush predators (Minton and McLean 1982; Diana 1983, 1987). In fact, the only thoroughly verified application of fish bioenergetics models under in situ conditions (Rice and Cochran 1984) involved largemouth bass which have repeatedly been shown to have low activity costs (Beamish 1970; Rice 1981; Rice et al. 1983). Rice and Cochran (1984) predicted largemouth bass growth and cumulative consumption within 8.5% of observed values.

Stewart and Binkowski (1986) attempted to account for the activity component in bioenergetics models applied to the alewife (*Alosa pseudoharengus*) by estimating activity costs via laboratory derived relationships between swimming speeds and water temperatures. In the lakes we studied water temperatures were very similar, the mean water temperature in 10 of the 12 lakes falling within a 2°C range (Boisclair and Leggett 1989a). While swimming speed/water temperature relationships are not available for perch, we seriously doubt that differences in activity induced by temperature differences could account for the among-population variability in activity costs we observed. Our analyses suggest that unless and until logistically practical methods are available for directly estimating in situ activity rates, the development of acceptably balanced bioenergetics models for actively foraging fishes will be possible only in cases where reliable in situ estimates of growth *and* food consumption rates are available.

Our analyses support Kerr's (1982) hypothesis of a linear positive relationship between activity costs and feeding levels. We also demonstrated that the Kerr (1982) model adequately accounted for variability in activity costs. However, because of the important contribution of activity costs to the total bioenergetics budget, even small differences between predicted and observed activity costs lead to large errors in predicted food consumption levels.

The coherence between activity costs estimated using Kerr's (1982) model and those obtained by difference using the Kitch-

ell et al. (1977) model does suggest that further exploration of the relationship between activity and consumption rates is worthwhile. However, the demonstrated sensitivity of predicted food consumption estimates to variations, and/or errors, in activity rate estimates reaffirms the fact that caution must be exercised when applying the output of fish bioenergetics models in which both consumption rates and activity rates are unknown.

Accuracy and Precision of Predictions of Bioenergetics Models

Carline (1987) suggested that cumulative consumption estimates derived from bioenergetics models are more accurate than those derived through repeated point estimates derived using methods developed by Eggers (1977) and Elliott and Persson (1978). His reasoning was based on the belief that consumption estimates derived from bioenergetics models more effectively integrate feeding levels over time, thereby avoiding the perceived problem of major day-to-day variability in fish daily ration. In support of the latter assumption Carline referred to experiments involving 0+ largemouth bass (Smagula and Adelman 1982) which were offered excess food consisting of a single prey, the fathead minnow (*Pimephales promelas*). The size of this prey, relative to that of the predator, dictated that the consumption of a single prey item changed the daily ration estimate by 5% of wet body weight. This lead to a highly discontinuous frequency distribution of daily ration estimates. Moreover, because of the large prey size, the digestion of even a relatively small number of prey is likely to require more than 24 h. Hence, bass which obtained a full ration on one day are likely, a priori, to exhibit lower consumption the next. The general difficulty of estimating fish daily ration for piscivorous fishes has been highlighted by Diana (1983) and Rice and Cochran (1984). Consequently, the primary conclusion to be drawn from these studies is that traditional methods (Eggers 1977; Elliott and Persson 1978) are not effective for estimating food consumption for fish that irregularly consume large prey which require long digestion times. This limitation has long been recognized (Cochran 1979; Elliott 1979; Eggers 1979; Pennington 1985; Olson and Mullen 1986).

During the past 6 yr we have executed over 200 in situ experiments to determine daily ration for numerous planktivorous and benthivorous fish (Boisclair and Leggett 1985, 1988, 1989a, see also Nakashima and Leggett 1978). We found the extreme variations noted by Smagula and Adelman (1982) to be rare events. Bioenergetics models may, indeed, provide better estimates of cumulative consumption for piscivorous fishes. Our data clearly indicate, however, that this is not true for planktivorous and/or benthivorous fishes.

The difference between bioenergetics and traditional approaches to the estimation of food consumption rates for planktivorous/benthivorous fish may be one of both precision and accuracy. Traditional methods for estimating fish daily ration typically require estimates of 2 variables: (1) the quantity of food found in the stomach or digestive tract of individual fish during the course of one day, and (2) the evacuation rate prevailing during that day (Eggers 1977; Elliott and Persson 1978; Pennington 1985; Olson and Mullen 1986; Boisclair and Leggett 1985, 1988). The error associated with daily ration estimates are in the order of 5–10% for laboratory determinations (Elliott and Persson 1978) and 15–25% for field determinations (Boisclair and Leggett 1988). The estimation of food consumption rates from bioenergetics models typically involves the use of approximately 20 model parameters (Stewart and Binkowski 1986), at least 2 (fish weight, water temperature) and, in some cases up to six more (contribution of prey type to fish diet, undigestible fraction of food consumed, caloric content of the food, etc.; see Hewett and Johnson 1987) model variables each of which has an associated experimental and measurement error. These errors, plus the undetermined error related to assumptions regarding the ratio of active to standard metabolism or in situ fish swimming speeds, render evaluation of the 95% confidence interval around any prediction of bioenergetics models difficult. Monte Carlo simulations executed by Bartell et al. (1986) suggest that predictions of bioenergetics models must vary at least 2 fold for statistically significant difference to be detected. This contrasts sharply with our finding that 2 field derived estimates of food consumption, developed using the Eggers (1977) model, that vary by as little as 35% (Boisclair and Leggett 1985, 1988, 1989a) can be effectively discriminated.

This problem of precision applies not only to the prediction of food consumption rates using the Kitchell et al. (1977) or Kerr (1982) models, but also to estimates of activity rates determined from their models. Both our Monte Carlo simulations and the magnitude of the among-population variability in activity rates suggest that the differences we observed are statistically and biologically significant.

The Ecological Importance of Activity Costs

We recently suggested that energy costs related to activity might represent one of the most important determinants of among-population variability in fish growth rates (Boisclair and Leggett 1989a,b,c). This suggestion was based upon the observed susceptibility of activity costs to environmental influences such as water temperature, prey mobility, prey distribution, prey availability; to the presence of other fish (via social stress) (Kerr 1971a,b; Ware 1975; Minton and McLean 1982; Weisberg and Lotrich 1982; Adams et al. 1982; Medland and Beamish 1985; Lemons and Crawshaw 1985; East and Magnan 1987); and to the lack of simple relationships between fish growth and food consumption as revealed in our studies (Boisclair and Leggett 1989a,b,c). We further suggested, on the basis of the strong and consistent negative relationships between fish growth and fish density revealed by our studies (despite the frequent absence of relationships between the quantity or quality of food consumed and fish density), that non-exploitative interactions between fish may be a major contributor to among-population variability in fish growth (Boisclair and Leggett 1989c). Because the activity costs reported here were determined, in part, from our field derived growth and food consumption rates, it is not possible to execute a statistically independent assessment of the expected negative relationship between growth rates and activity costs, or of the expected positive relationship between activity rates and fish density. However, the magnitude of, and the among-population variance in, the quantity of energy allocated to activity in the perch we studied is consistent with our hypothesis that this component of the bioenergetics budget of fishes has the potential to contribute in an important way to the explanation of inter-population differences in perch growth and, by extension, to the variance in growth of other actively foraging fish species.

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Triacylglycerol Content as a Condition Index for Fish, Bivalve, and Crustacean Larvae

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The lipid biochemistry of environmentally stressed larval fish, bivalves and crustaceans is presented to illustrate the utility of a larval condition index based on lipid composition. Larvae under environmental stress are often unable to obtain sufficient energy from exogenous sources and, as a result, endogenous energy reserves are catabolised to maintain basal metabolism. The storage lipid triacylglycerol (TAG) is of particular importance in this respect, TAG content consequently correlating with the physiological condition of a larva. However, absolute TAG content cannot be directly correlated with larval condition because of its dependency on larval size. It is proposed that TAG content can be correlated with larval condition when expressed in the form of a TAG-sterol ratio that accounts for the size dependency of TAG content. This proposition is supported by data that illustrate a highly positive correlation between sterol content and dry weight for larval herring and larval American lobster. Examples of TAG-sterol ratios are calculated from a survey of studies relating to the lipid class composition of nutritionally and pollutant stressed larvae of some marine fish and crustaceans.

La biochimie des lipides chez les larves de poissons, de bivalves et de crustacés exposées à un stress environnemental est décrite afin de montrer l'utilité d'un indice d'état des larves fondé sur la composition en lipides. Les larves exposées à un stress environnemental sont souvent incapables de satisfaire leurs besoins en énergie à partir de sources exogènes de telle sorte qu'elles doivent cataboliser leurs réserves énergétiques endogènes afin de maintenir leur métabolisme basal. Le triacylglycérol (TAG), un lipide de réserve, joue à cet égard un rôle important de telle sorte que la quantité de TAG dont disposent les larves est corrélée avec l'état physiologique de celles-ci. Cependant, on ne peut pas établir de corrélation directe entre le contenu en TAG en valeur absolue et l'état des larves, car le contenu en TAG dépend de la taille des larves. Pour obtenir une corrélation directe, nous proposons d'exprimer le contenu en TAG sous la forme d'un rapport TAG-stérol qui prendrait en compte le fait que le contenu en TAG dépend de la taille des larves. Cette proposition est fondée sur des données indiquant l'existence d'une corrélation positive forte, observée chez les larves de hareng et les larves de homard d'Amérique, entre le contenu en stérol et le poids sec. Nous avons obtenu certains rapports TAG-stérol en effectuant des calculs à partir de données tirées d'études portant sur la composition des lipides suivant leur classe chez certaines larves d'espèces de poissons et de crustacés marins exposées à un stress nutritionnel ou causé par des polluants.

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An approach to understanding the influence of physical, chemical, and biological oceanographic conditions on the feeding ecology of larval marine organisms necessitates that the physiological condition of larvae be readily and accurately determined. Larval condition is primarily influenced by food availability, but is also dependent on variables such as temperature and pollutant stress that can increase metabolic activity, in turn increasing the energetic demands of a larva. Morphological, histological, and histochemical methods of assessing condition in fish larvae have been successfully applied in field studies (Shelbourne 1957; O'Connell and Paloma 1981; Hewitt et al. 1985; Theilacker 1986). However, considerable attention has recently been given to biochemical means of condition assessment using RNA–DNA ratio (Buckley 1984; Clemmesen 1987), digestive enzyme content (Pedersen et al. 1987) and lipid composition (Martin et al. 1985; Fraser et al. 1987). Condition assessment based on RNA–DNA ratios has been most thoroughly researched, having been applied in field studies (Buckley and Lough 1987; Robinson and Ware 1988).

This paper proposes a novel biochemical condition index for the larvae of fish, bivalves, and crustaceans that is based upon lipid composition. The biochemical basis of the method is presented and is followed by examples collected from a survey of studies on the lipid biochemistry of nutritionally and pollutant stressed larvae of marine fish and crustaceans.

Lipid Biochemistry of Larval Fish, Bivalves, and Crustaceans

The early larval development of many marine organisms is characterised by a post-hatching period during which larvae are largely dependent upon endogenous energy reserves. Invariably, much of this endogenous energy is in the form of triacylglycerol (TAG), the major depot or storage lipid in animal cells (Lehninger 1975). Triacylglycerol is catabolised by pre-feeding, yolk-sac larvae of Atlantic salmon, *Salmo salar* (Cowey et al. 1985), Atlantic herring, *Clupea harengus* L. (Fraser et

al. 1987), European sea bass, *Dicentrarchus labrax* (Quessada and Pionetti 1987), Atlantic cod, *Gadus morhua* L. (Fraser et al. 1988), American lobster, *Homarus americanus* (Sasaki 1984), and several bivalve species (Gallager et al. 1986). Triacylglycerol continues to be depleted during the early stages of larval development, until the energetic demands of growth and metabolism are met from exogenous sources.

When exogenously derived energy ultimately exceeds the immediate metabolic demands of a larva, then excess energy can be stored as TAG. This was observed in enclosure-reared Atlantic herring larvae growing at 0.22 $mm \cdot d^{-1}$ (Fraser et al. 1987), and in laboratory-reared plaice (*Pleuronectes platessa*) larvae (Ehrlich 1974a). In contrast, when exogenously derived energy is insufficient to maintain the basal metabolism of the larvae of these species, endogenous TAG is catabolised (Ehrlich 1974a,b; Fraser et al. 1987). A similar relationship between TAG content and nutritional condition is expected for post-yolk-sac larvae of other fish, given that TAG is a ubiquitous form of energy storage in the eggs, (Kaitaranta 1980; Kaitaranta and Ackman 1981; Brind et al. 1982; Tocher and Sargent 1984; Falk-Petersen et al. 1986), yolk-sac larvae (Eldridge et al. 1982; Vetter et al. 1983; Fraser et al. 1988) and adults (Love 1980) of many fish species.

The accumulation of TAG by well-nourished larval Atlantic herring occurs throughout their development. However, during metamorphosis the rate of TAG deposition is notably reduced, although absolute levels of TAG do not decline at this time (Fraser 1986). Following metamorphosis, the rate of TAG accumulation increases markedly, resulting in juvenile herring with a high TAG content similar to that of adults at the end of the summer feeding season (Iles and Wood 1965). Fukuda et al. (1986) obtained similar results for Pacific herring *Clupea pallasi*, and demonstrated that reduced TAG accumulation during metamorphosis is accompanied by a continuously declining protein-DNA ratio that was considered indicative of cell proliferation. Metamorphosis in herring appears, therefore, to be characterised by a change in metabolism whereby the high rate of TAG accumulation achieved during the latter stages of larval development is reduced, with a higher proportion of exogenously derived energy probably fueling the physiological changes associated with metamorphosis (Blaxter and Jones 1967; Blaxter et al. 1981; Batty 1984). Consequently, if TAG content is to be correlated with the nutritional condition of late developmental stages of larval fish, potential effects of metamorphosis on TAG content must be considered.

Well-nourished American lobster larvae also accumulate TAG, generally in an exponential manner during development through stages I to IV (Sasaki 1984; Sasaki et al. 1986) , with TAG being catabolised under starvation conditions (Sasaki 1984). The molting of these larvae is sometimes associated with the depletion of TAG, probably reflecting a reduction in food intake at this time (Sasaki 1984; Sasaki et al. 1986). Thus, while the TAG content of larval American lobster is influenced by nutritional condition, the effects of molting on TAG content in pre- and post-molt stages must be considered. Triacylglycerol can be expected to represent an important energy reserve in the larvae of other crustaceans, given the role of this lipid as an energy reserve in the juveniles and adults of other species (Heath and Barnes 1970; New 1976; Teshima et al. 1977).

Triacylglycerol accumulation during feeding, and starvation-induced depletion, also occurs in the larvae of several bivalve species, (Holland 1978; Gallager and Mann 1986a; Gallager et al. 1986), and in the larvae of other benthic marine invertebrates (Holland 1978). Larval *Ostrea edulis* also catabolise a large proportion of endogenous TAG during metamorphosis (Holland and Spencer 1973), reflecting the inability of some bivalves to feed at this time (Bayne 1965; Hickman and Gruffydd 1971). The basis for TAG accumulation by larval *Ostrea edulis* is readily apparent given the extent of the reliance on endogenous energy during metamorphosis (Holland and Spencer 1973). The rationale for TAG accumulation by larval fish and American lobster larvae is less obvious, although TAG is sometimes catabolised to minor degrees during molting in this crustacean (Sasaki 1984). Most probably, the accumulation of TAG by the larvae of these species primarily represents the formation of a readily accessible source of energy that can be utilised during periods of food shortage, thus buffering against potentially high variability in food availability.

Determination of TAG Content

Gallager and Mann (1986a) developed a rapid and inexpensive technique for the quantification of neutral lipid, specifically TAG (Gallager et al. 1986), in bivalve larvae that was based on the staining and photodensitometric quantification of oil droplets. This method is unsuitable for specimens in which TAG is not present as discrete droplets. A suitable alternative method of TAG quantification involves the use of the Iatroscan TLC-FID system (Ackman 1981; Fraser et al. 1985). This approach was successfully applied in studies of the lipid composition of composite samples containing 140–500 larvae of Atlantic herring (Fraser et al. 1987) or Atlantic cod (Fraser et al. 1988), and has been used successfully on a more sensitive scale for the quantification of as little as 0.5 μg of TAG in *Calanus pacificus* (Hakanson 1984). This method is applicable to the study of single fish larvae; a 6-d-old cod larva containing 0.9 μg of TAG (Fraser et al. 1988). A further major advantage of using the Iatroscan is that free fatty acids (FFA) are simultaneously quantified, high FFA levels indicating the occurrence of lipid hydrolysis during improper storage or processing. If hydrolysis has occurred, TAG content cannot reliably be used as a measure of condition because of the potential loss of some of this lipid (Sasaki and Capuzzo 1984).

Absolute and Relative Expression of TAG Content

The TAG content of a larva cannot represent a direct indicator of condition because of the dependency of TAG content on larval size (Gallager and Mann 1986b), and should be expressed relative to body size or dry weight. A similar method of relative expression has been devised for RNA content, the use of RNA–DNA ratios accounting for the size dependency of RNA content (Bulow 1987).

It is often impractical to determine the dry weight of individual larvae of aquatic organisms prior to determining their TAG content given their small size and the potential for lipid hydrolysis and oxidation during freeze-drying and weighing. Alternatively, TAG content could conceivably be expressed relative to that of another lipid, the amount of which correlated highly with larval size. Sterols serve as precursors of steroid hormones (Lehninger 1975), including ecdysteroids in crustaceans (Chang and Bruce 1981; Soumoff and Skinner 1983), but are quantitatively most involved as structural components of cell membranes (Nes 1974). The finding that sterols represent

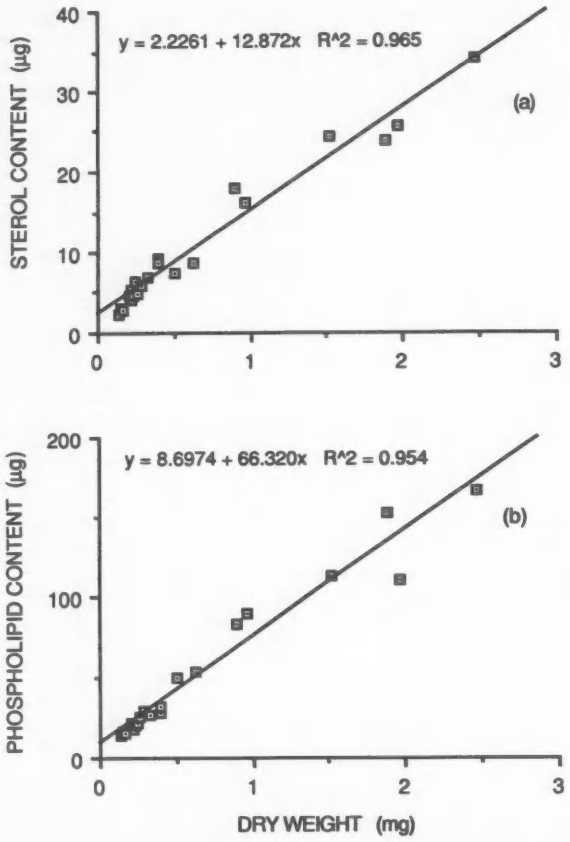

FIG. 1. Weight of sterols (a) and total phospholipids (b) versus dry weight for enclosure-reared Atlantic herring larvae with growth rates ranging from 0.05 to 0.24 mm·d^{-1}.n = 21. Calculated from data in Fraser (1986) and Fraser et al. (1987).

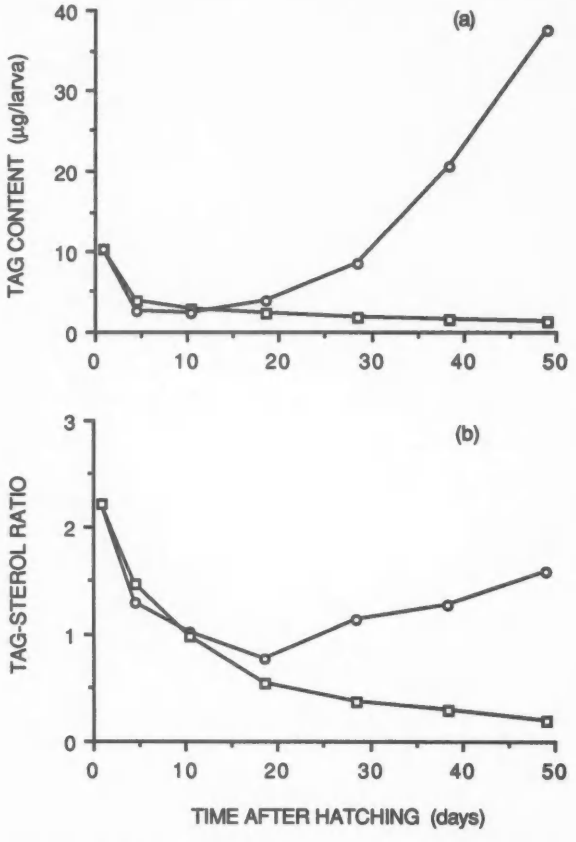

FIG. 2. Triacylglycerol content (a) and triacylglycerol-sterol ratios (b) for enclosure-reared Atlantic herring larvae with growth rates averaging 0.05 (□) and 0.24 mm·d^{-1} (○). Data from Fraser (1986) and Fraser et al. (1987).

a remarkably constant proportion of the wet weight of a wide range of eukaryotic organisms (Nes 1974) is consistent with their predominantly structural role. The evaluation of data from Fraser et al. (1987) shows that sterol content has a high positive correlation with the dry weight of larval Atlantic herring that had growth rates ranging between 0.05 and 0.24 mm·d^{1}, and differences in dry weight of over one order of magnitude (Fig. 1a). Thus, in this instance, TAG content can be expressed relative to that of sterol content to yield a ratio that accounts for the size dependency of TAG content (Fig. 2). The total phospholipid content of these same herring larvae is also highly correlated with dry weight (Fig. 1b), but sterol content is considered a superior measure of larval size due to the relative ease in quantifying this lipid class. Sterol and TAG content can be determined simultaneously with the Iatroscan following development in a non-polar solvent (Fraser et al. 1985), while the use of an internal standard permits the absolute quantification of these two lipids (Hakanson 1984). Phospholipids must be analysed separately following development of the lipid sample in a more polar solvent system because total phospholipids are overestimated when quantified as a total phospholipid peak following a neutral lipid development (Fraser et al. 1985).

The sterol content of American lobster larvae is also highly correlated with dry weight (Fig. 3a). The sterol content of stage I and II larvae remains essentially unchanged after 5–6 d of starvation (Sasaki 1984), illustrating the independence of sterol content and nutritional condition. Greater variability in the amounts of total phospholipid (Fig. 3b) are a consequence of the catabolism of both phosphatidylcholine and phosphatidylethanolamine during starvation (Sasaki 1984), thus favouring the selection of sterols as a measure of larval size. With the rapid catabolism of TAG during starvation (Sasaki 1984), these changes in lipid class composition are reflected in declining TAG-sterol ratios (Fig. 4). Triacylglycerol-sterol ratios generally increase throughout development in larval American lobster (Fig. 5), due primarily to the rapid accumulation of TAG, with minor inflexions at each molting due to reduced TAG deposition (Sasaki 1984). It is noteworthy that TAG content can additionally be expressed relative to the readily identifiable stages and substages through which larval lobsters develop (Aiken 1980; Sasaki 1984).

No data exist that express the sterol content of nutritionally stessed bivalve larvae, although they rapidly catabolise TAG when nutritionally stressed (Holland and Spencer 1973; Gal-

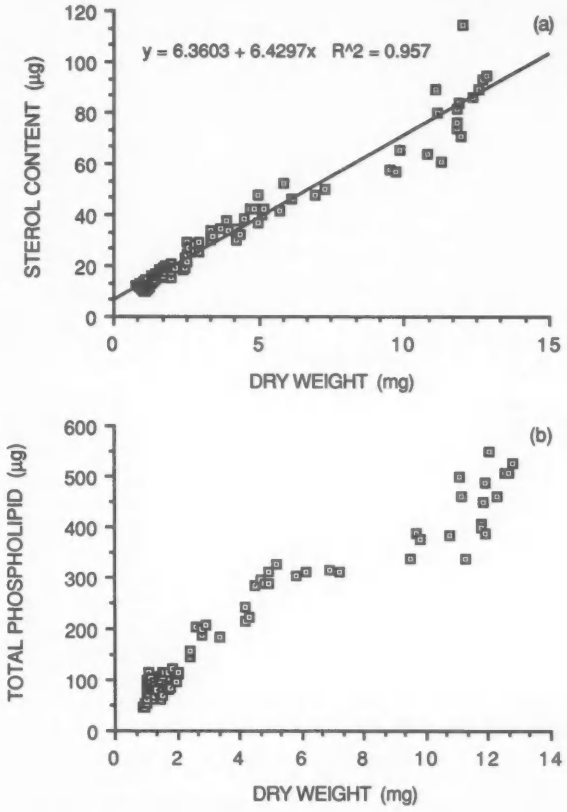

FIG. 3. Weight of sterols (a) and total phospholipids (b) versus dry weight for fed stage I to IV, and starved stage I and II American lobster larvae. $n = 114$. Calculated from data in Sasaki (1984).

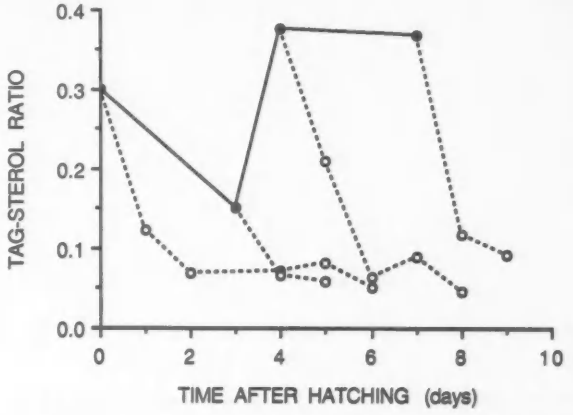

FIG. 4. Triacylglycerol-sterol ratios for fed (●) and starved (○) stage I and II larvae of American loster. Calculated from data in Sasaki (1984).

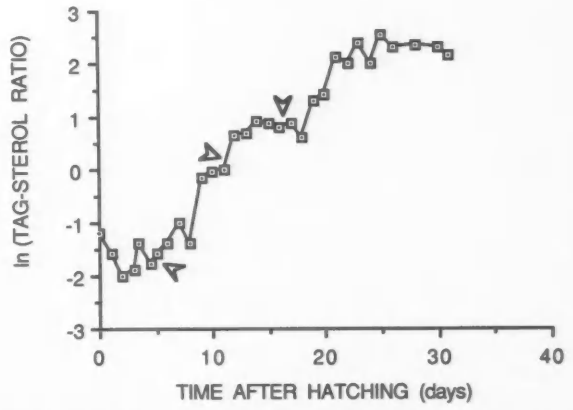

FIG. 5. Triacylglycerol-sterol ratios during development from stages I to IV for fed American lobster larvae. Note the natural log scale. Arrow-heads indicate time of molting. Calculated from data in Sasaki (1984).

lager et al. 1986). It is, however, to be expected that sterol content will correlate highly with the dry weight of the larvae of a wide range of bivalve species, in which case a condition index based on TAG-sterol ratios should be widely applicable.

TAG Content and Pollutant Stress

A TAG-based condition index is applicable to the study of any form of stress that results in the catabolism of endogenous TAG. Exposure to petroleum hydrocarbons can lead to reduced food intake and altered metabolism in various aquatic organisms (Capuzzo et al. 1984; Widdows et al. 1987), resulting in increased catabolism of endogenous energy reserves. Decreased TAG-sterol ratios were calculated from the data of Capuzzo et al. (1984) for American lobster larvae exposed to 0.25 ppm South Louisiana crude for 96 h (Table 1). In this case, differences were due to both decreased TAG content and increased sterol content of oil-exposed specimens. These apparent effects of oil exposure on sterol content illustrate that if TAG-sterol ratios are to be applied to the larvae of any species, their lipid biochemistry must be thoroughly understood.

Juvenile blue crabs *Callinectes sapidus* exposed to South Louisiana crude oil catabolised endogenous TAG at a rate that appeared directly related to exposure concentration (Wang and Stickle 1988). Sterol content was relatively unaffected in exposed specimens, resulting in a marked reduction in TAG-sterol ratios (Fig. 6). Differences in TAG-sterol ratios calculated for control and oil-exposed crabs were often considerably greater than differences in the RNA-DNA ratio of similarly treated specimens (Wang and Stickle 1988). These changes in

TABLE 1. Triacylglycerol-sterol ratios for larval American lobster exposed for 96 h to 0.25 ppm South Louisiana crude oil-water dispersions. Calculated from data in Capuzzo et al. (1984).

	Triacylglycerol-sterol ratio	
Larval stage	Control	Oil-exposed
I	0.55	0.40
II	2.27	1.58
IV	7.61	5.76

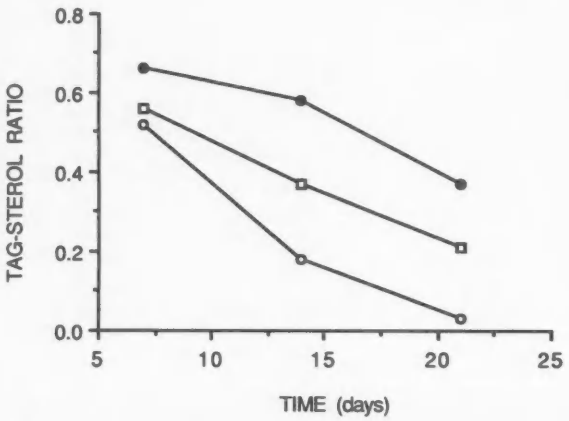

FIG. 6. Triacylglycerol-sterol ratios for juvenile blue crabs exposed to petroleum hydrocarbons at concentrations of 0 (●); 620 (□) and 2011 (○) parts per billion. Calculated from data in Wang and Stickle (1988).

TAG-sterol ratio in juvenile crabs illustrate that the biochemical principles relating to the role of TAG as an energy store can apply to older as well as larval stages of aquatic organisms.

Application of TAG-Sterol Ratios

If TAG-sterol ratios derived from laboratory-reared larvae are to be applied in the field, a method of intercalibration is required. Blaxter (1976) showed that over a range of developmental stages, fat content in several fish species was consistently higher in reared than in wild specimens. Ehrlich (1975) also found that starved, post-yolk-sac larvae of herring and plaice held in a laboratory had a higher TAG content than wild larvae of similar size. Fraser et al. (1987) demonstrated that TAG catabolism was more pronounced in pre-feeding larval herring placed in the presence of potential prey organisms in 300 m^3 enclosures than in equivalent larvae held in the laboratory in an 18-L container containing no food. These observations serve to illustrate the risks in indiscriminately applying TAG-sterol ratios, or any other condition index, that has been calibrated using laboratory reared larvae.

An alternative approach to laboratory calibration for fish larvae would be to determine the TAG-sterol ratio at the time when the extent of exogenous food intake begins to influence TAG content. This could be considered a threshold value, with subsequently increasing or decreasing values being indicative of 'good' or 'bad' larval condition, respectively. This threshold TAG-sterol ratio could be calculated in the laboratory using large-volume containers and by otherwide minimising the unnatural effects of laboratory conditions. Alternatively, it may in some instances prove feasible to sample discrete cohorts of larvae during the first two weeks after hatching and determine the threshold TAG-sterol ratio on the basis of the larval growth and feeding conditions observed. Knowing the TAG-sterol ratio at the point-of-no-return would also be of value in identifying non-viable larvae in the wild.

Conclusions

Considering the ubiqitous nature of TAG as an energy reserve, a condition index based on TAG content, with the complementary expression of a TAG-sterol ratio, appears to be a useful method for determining the physiological condition of the larvae of a range of aquatic species. Triacylglycerol content is directly affected by exogenous food intake, but is also influenced by forms of environmental stress that increase metabolic activity and reduce food intake. A knowledge of TAG content is also of predictive value, permitting an estimate of the time a larva might be expected to survive a period of food limitation. The Iatroscan TLC-FID method of lipid class analysis is suitable for the qantification of TAG and sterols in single fish larvae, the simultaneous determination of free fatty acids being of value in verifying proper sample storage and processing.

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Establishment of a Bedrock Epilithic Community in a Small Stream: Microbial (Algal and Bacterial) Metabolism and Physical Structure

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Stock, M. S., and A. K. Ward. 1989. Establishment of a bedrock epilithic community in a small stream: microbial (algal and bacterial) metabolism and physical structure. Can. J. Fish. Aquat. Sci. 46: 1874–1883.

Patterns of epilithic algal and bacterial productivity were examined in a developing community on newly exposed stream bedrock for a period of 10 wk and in an undisturbed bedrock community used as a seasonal control. Physical and chemical changes were minimal over the experimental period. Bacterial colonization occurred initially and was rapidly followed by the development of a monolayer of adnate diatoms. Subsequent bacterial development coupled with maximum rates of bacterial productivity may have depended upon the algal cells for physical refugia, mucilage production, and/or other growth-promoting substrates present in algal photosynthate. After the diatom monolayer, filamentous algae developed despite the presence of high densities of snail grazers. By the end of the experiment, community composition on both substrata was generally similar although filamentous blue-green algae were a more important component of the native communities. Communities on newly exposed rock had higher total levels of epilithic productivity than on native rock where bacterial numbers averaged 2.26×10^{11} cells/m^2. On native rock epilithic bacterial productivity averaged 72 mg C·m^{-2}·d^{-1}, yielding an average turnover time of 0.56 d; algal productivity averaged 224 mg C·m^{-2}·d^{-1}. These data suggested that epilithic production was not quantitatively limiting as a food resource for grazing snails in this stream during the summer months.

La productivité de bactéries et d'algues épilithiques a été observée sur une période de 10 sem dans une communauté naissante sur un lit rocheux exposé depuis peu et dans une communauté établie sur un substrat rocheux non perturbé (substrat de référence saisonnier). Les modifications physiques et chimiques ont été peu marquées au cours de la période d'observation. La colonisation bactérienne s'est produite en premier et a été rapidement suivie par la croissance d'une monocouche de diatomées adnées. La prolifération bactérienne subséquente, associée à des taux maximums de productivité bactérienne, pourrait avoir été liée à la présence de cellules d'algues, qui leur assurent un refuge physique, la production de mucilage et/ou un autre substrat favorable à la croissance présent dans les photosynthétats des algues. Après les diatomées, des algues filamenteuses se sont développées, malgré la présence d'un grand nombre d'escargots brouteurs. À la fin de la période d'observation, la composition des communautés sur les deux substrats était généralement semblable mais les algues bleues filamenteuses formaient une composante plus importante des communautés plus anciennes. Les communautés sur les roches nouvellement exposées présentaient des niveaux totaux de productivité épilithique plus élevés que dans l'autre cas, où le nombre de bactéries était en moyenne de $2{,}26 \times 10^{11}$ cellules/m^2. Sur le substrat de référence, la productivité de bactéries épilithiques était en moyenne de 72 mg C·m^{-2}·j^{-1}, avec un temps moyen de renouvellement de 0,56 j; la productivité d'algues était en moyenne de 224 mg C·m^{-2}·j^{-1}. Ces données semblent indiquer que la production épilithique n'introduisait pas de limite quantitative à l'alimentation des escargots brouteurs dans ce cours d'eau au cours de l'été.

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In recent years, a major tenet of stream ecology has been that allochthonous organic matter provides the energy base that drives small forested, headwater stream ecosystems; autochthonous input by primary producers in these ecosystems has been considered less important (Fisher and Likens 1973; Cummins 1974). However, autochthonously produced organic matter, in spite of being a small proportion of total organic inputs, may be important to the production of some secondary producers. Recent evidence for this has been provided in Bear Brook (Mayer and Likens 1987), where 75% of the production of a caddisfly was supported by autochthonous organic matter.

In streams dominated by bedrock and cobble substrata, the biofilm attached to rocks, i.e., epilithon (Lock 1983), can provide an important food source for micro-and macroinvertebrates (Rounick et al. 1982; Rounick and Winterbourn 1983; Winterbourn et al. 1985). This epilithic community mainly consists of autotrophs (algae) and heterotrophs (bacteria) embedded in a polysaccharide matrix derived from these organisms (Lock et al. 1984). This complex community may entrain nutrients and substrate molecules, and extracellular enzymes produced by the biota may facilitate utilization of these compounds (Hoppe 1984). Lock (1983; et al. 1984) has proposed a model of this community in which the close physical proximity of autotrophs and heterotrophs allows for efficient cycling of CO_2, O_2, and nutrients between trophic levels within the matrix. An understanding of the interactions of the various

components of the biofilm and productivity of the organisms is necessary in order to evaluate their importance to the ecosystem (or other trophic levels).

The assumption of a mutual dependence between epilithic algae and bacteria is the basis for Lock's model and may be used to explain the high productivity of this community in spite of grazing by invertebrates and stochastic events such as scour by floods. Strong bacterial/algal interactions have been illustrated in planktonic systems where bacteria utilize dissolved organic carbon originating from dead or dying phytoplankton or secreted from actively growing algal cells (Cole 1982; Coveney 1982; Brock and Clyne 1984). Few stream studies have focused on biotic interactions between algae and bacteria (Haack and McFeters 1982a; 1982b). No stream studies have compared both epilithic algal and bacterial productivity over time in order to establish the relative patterns of each. Disruption of the physical integrity of the biofilm communities has been shown to change the physiology of the biotic components being measured (Murray et al. 1987). Therefore, it is important to maintain the integrity of the community, i.e., keep it attached to the underlying substratum, when measuring its activity.

The goal of this study was to examine the productivity and composition of the microbial components of an intact epilithic community. We did this by following the reestablishment of the epilithic community on bedrock from which the surface layer of rock had been removed (new rock or NR). Total and cell-specific algal and bacterial productivity and composition on NR over time were compared to a nearby undisturbed bedrock community (old rock or OR). Monitoring the colonization of NR has the potential for showing how the heterotrophic and autotrophic components establish themselves in relation to each other. Simultaneously following the microbial productivity and composition of communities on undisturbed rock yields a control and allows a comparison which indicates when the NR community has reached "maturity", i.e., when it is similar to OR. Specifically the research in this study was designed to address the following questions: What is the pattern of epilithic bacterial and algal physical structure and activity, and how do these values compare with estimates from other studies in this and other systems? How do patterns of phototrophic (algal) and heterotrophic (bacterial) activity compare as the microbial community develops on rock substratum and do these patterns reflect potential interactions between trophic levels?

We hypothesized that autotrophic (algal) production would be greater than heterotrophic (bacterial) production on bedrock in spite of the fact that the study was conducted in a forested headwater stream. We also predicted that bacterial production would be a relatively consistent proportion of algal production, and that patterns of algal and bacterial recovery would show strong parallel trends if these trophic levels were linked.

Materials and Methods

Study Site

Little Schultz Creek is a small second order moderately alkaline stream located 48 km southwest of Birmingham, Alabama, in a forested watershed (Bibb County, Sec. 20, T24N, R10E). This stream supports a relatively high biomass of snails with mean densities from 590 to 790/m^2 during the summer months (Richardson et al. 1988). The study site for this research was a 2.5-m bedrock riffle. This site was located within a 250-m reach of stream with limestone and dolostone bedrock substratum in riffles alternating with cobble and sandy pools that have been described previously (Lay and Ward 1987; Stock et al. 1987). Seven patches of bedrock, each approximately 40 cm^2, were cleared by removing the surface of the rock with a rock hammer on 5 July 1986. Current over all 7 patches was a uniform 35 cm/s. At intervals of 5 d, 1, 2, 4, 7 and 10 wk (final sampling date: 13 Sept. 1986), one sample each of NR and OR was removed with a rock hammer and used to determine algal and bacterial standing stock, algal composition, and bacterial and algal productivity by methods described below. The OR provided a control by which seasonal changes over the 10-wk experimental period could be assessed.

In a preliminary experiment (4 July 1986) light readings (LI-COR 185-B) were taken every 30 min from 7:30 AM to 6:00 PM at 7 points across the riffle to insure uniform light among cleared areas. Over the test sites light input was uniform with peak light input occurring from 11 AM to 1 PM CDT. Current (Marsh McBirney 201-M Flowmeter), temperature, 20°C (mercury thermometer), pH, 7.2 (Orion 407A), and alkalinity, 170 mg($CaCO_3$)/L (titration with 0.02 N H_2SO_4 to an endpoint of pH 4.5) were also uniform over the test sites (Wetzel and Likens 1979). These parameters, with the exception of current, were determined at midriffle on each subsequent sampling date. Weather data for the duration of the experiment were collected at Centreville, Alabama, approximately 7 km from the study site, and provided by the National Oceanographic and Atmospheric Administration in Asheville, NC.

Algal Biomass and Productivity

The methods used to measure primary productivity were similar to those used previously (Ward et al. 1985; Stock et al. 1987). Bedrock fragments (each approximately 25 cm^2) with intact, attached microbial communities, both OR and NR, were placed in a 2-L clear Plexiglass chamber in which water recirculated. Ten mL of ^{14}C-$NaHCO_3$ (1.4×10^{-16} Bq/mL, New England Nuclear) were added to the chamber, which was incubated in the stream for 2–4 h. Samples were then removed, rinsed with nonradioactive stream water, refrigerated, and transported to the laboratory. Within 2 h of incubation, all samples were scraped with a toothbrush into a known volume of water to form a pooled sample. Subsamples of each scraped, pooled sample, one for OR and one for NR, were then filtered onto 0.22 μm pore size cellulose acetate filters (Millipore).

For total primary productivity, filters were dried, fumed in HCl for 10 min, converted to CO_2 in a Packard oxidizer (Model 306), collected in a scintillation cocktail, and radioassayed in a Beckman liquid scintillation counter (Model LS 3801). Values were corrected for oxidation efficiency (always greater than 99%). Activity in dpm was converted to mg carbon fixed·m^{-2}·h^{-1} using the known quantity of radioisotope added as well as the quantity of inorganic carbon available from measurements of water temperature, pH, and alkalinity (Wetzel and Likens 1979). A previous study in Little Schultz Creek indicated that there was <2% ^{14}C uptake in dark chambers compared to uptake in light chambers (Stock et al. 1987). Therefore, dark chambers were not used in this study. Radiant energy incident on the stream surface (watts/m^2) for each sampling period was measured using a LI-COR 55 printing integrater and pyranometer sensor. Daily production was calculated from hourly rates using the proportion of the incident light for the incubation period of the total light for a dawn to dusk period measured at the beginning of the experiment. Light input over

the incubation period on each sampling date was 53–79% of total daily light input.

The area of the top of the rock sampled was determined by weighing aluminum foil that was applied to the rock and cut out in the shape of the rock surface. Since aluminium foil is uniform in thickness, the weight of a piece of foil is directly proportional to its surface area. Pieces of foil of known area were weighed to obtain a conversion factor for calculating area from the weight of foil cut out from the surface of the rock.

Autoradiography was used to determine the cell specific activity of algal taxa and samples prepared by this procedure were used for determination of algal numbers and biovolumes. This procedure closely followed that of Paerl (1974). Aliquots of the scraped pooled sample were filtered onto cellulose acetate (Millipore HA) filters that were immediately frozen over liquid nitrogen. Frozen filters were then lyophilized and dried filters were stained with erythrosin B (3% in 5% phenol), destained for 2 h in distilled water and allowed to dry. Stained filters were glued to slides coated in albumin and were cleared by holding them over acetone fumes for no more than 5 s. These were then coated in Kodak NTB-3 liquid emulsion (diluted 1:1 with sterile distilled water), dried overnight in the dark and the emulsion exposed for 4–6 d at 5°C before being developed in Kodak D-19 developer (Brock and Brock 1968). Nonradioactive controls were processed by the same procedure and examined for background exposure and/or chemography.

Dried slides were examined with immersion oil applied directly to the autoradiograph at 1000× with a Zeiss IM microscope. No attempt was made to quantify different degrees of activity of algal cells. After 4 d exposure, active cells were at least 1/3 covered by silver grains; silver grains did not exceed background levels on inactive cells. A minimum of 200 fields/filter and 3 filters/sample were examined; generally a total of at least 800 fields was examined. Standard errors were calculated, but represent within-sample variation since all filters were subsamples of a single pooled sample; values for n are the number of filters or subsamples. It was not often possible in autoradiographs to determine the species of each cell; therefore, the algae were grouped into the most appropriate taxonomic categories. More specific identifications were made from samples preserved in 5% formalin. Experimental samples for 5 July (NR) and 13 Sept. (NR and OR) were lost.

Biomass was determined using measurements of cell sizes and calculations of biovolume based on cell shape (Pip and Robinson 1982; Steinman et al. 1987). A minimum of 20 cells of each taxon was measured to determine biomass from cell biovolumes and total numbers of cells as counted from slides prepared for autoradiography using a Whipple grid. Results were reported in the biovolume of active cells and of all cells in a given sample. Relative activity was calculated from the biovolume of active cells of a taxon divided by the total number of cells of that taxon.

Bacterial Biomass and Productivity

Bacterial production was estimated from [methyl-^{3}H]-thymidine (^{3}H-TdR) incorporation into DNA by modification of the method described by Findlay et al. (1984). Three to five bedrock chips were placed in each of 4 sterile 25-mm glass test tubes containing 20 mL of stream water. To each tube was added 2.7×10^{-15} Bq of ^{3}H-TdR (specific activity 210×10^{-9} Bq/mmole; ICN Radiochemicals). Tubes were then capped and incubated in the stream for 15 min. The incubation was terminated by washing the rocks 4× with 5% formalin. Preliminary experiments showed that uptake was optimum at 15 min and with 68.5 nM concentration of TdR. Additions of nonradiolabeled TdR did not decrease activity, therefore there was no apparent isotope dilution. Both light and dark tubes were incubated on each date and controls were killed with 5% buffered formalin. Since no consistent differences between light and dark treatments were observed, these were treated as replicates.

Nucleic acids were extracted by adding 20 mL of 0.3 N NaOH with 25 mM EDTA and 0.2% sodium dodecyl sulfate directly to the rocks. After a 9 h extraction period, the solution was removed from the rocks and labeled macromolecules were precipitated by adding an equal volume of 10% trichloroacetic acid (TCA) to chilled and neutralized samples. Carrier yeast *t*-RNA (BRL) was added to aid the precipitation of nucleic acids. The precipitate was then centrifuged (20 000 × *g*, 10 min) washed with cold 5% TCA, and DNA was hydrolyzed at 95°C for 30 min. After centrifugation (1 500 × *g*, 6 min), aliquots of the supernatant were added to Aquasol 2 (NEN) scintillation cocktail and radioassayed as above. The quantity of ^{3}H-TdR incorporated into DNA was calculated from activity in dpm by subtracting killed controls and converting dpm to nmoles incorporated from the quantity of TdR added and its specific activity.

Bacterial activity in stream water samples overlying rock chips was determined on 8 Aug. 1986 by adding 1.4×10^{-15} Bq ^{3}H-TdR to 10 mL of stream water incubated in the light for 15 min. The extraction procedure for DNA followed that of Fuhrman and Azam (1982). Bacterial activity in water alone was only 1.2% of epilithic bacterial activity.

Rock fragments for bacterial counts were scraped with a sterile toothbrush and collected in filter-sterilized water. Bacterial cells were counted from rock scrapings stained with DAPI (4′6-diamidino-2phenylindole) for epifluorescence microscopy counts (Porter and Feig 1980). A minimum of 20 fields/sample were counted using a Leitz Diaplan microscope. Bacterial biomass was determined by the method used by Edwards (1987) in which micrographs of DAPI-stained cells are measured using an image analysis system. The width and length of 50 cells/sample were measured using an Image Technology Model 3000 Image Analyzer and biovolume was calculated by assuming rods were a cylinder capped by hemispheres and cocci were spheres. Spiral bacteria were divided into straight segments and treated as the sum of the volume of rods making up the segments. Two pooled measurements were obtained: one for NR samples and one for OR samples. These biovolumes were then combined with numbers and activity to calculate production in carbon.

A factor of 2×10^9 cells/nmole TdR incorporated into DNA was used to convert activity to cells produced (Moriarty 1984). Cell volumes were converted to carbon with a conversion factor of 2.2×10^{-13} gC/μm^3 (Edwards 1987). Biomass in mg C/m^2 could then be calculated from the average size of cells times the number of cells and production (mg C·m^{-2}·h^{-1}) calculated by multiplying the number of cells produced times the average carbon content of each cell. Hourly values were converted to daily values by multiplying them by 24. Since few studies have been done of bacterial production on solid surfaces, turnover times in days were calculated from B/P ratios in order to compare production in this study with studies in other systems.

SEM of Rock Surfaces

Specimens of rocks were prepared for SEM as described previously (Stock et al. 1987). All samples were preserved in 2.5%

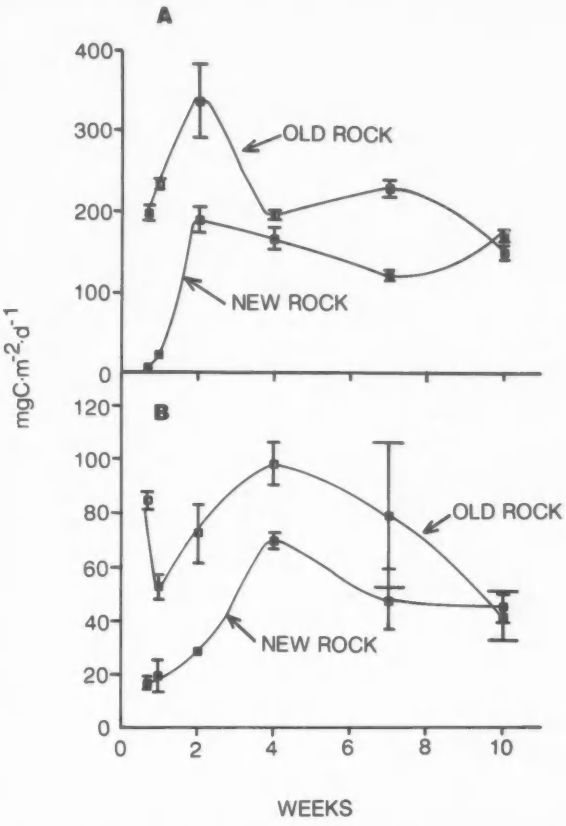

FIG. 1. A. Autotrophic (algal) and B. Heterotrophic (bacterial) microbial activity on OR and NR surfaces; bars indicate standard errors (SE) of the mean.

glutaraldehyde, post-fixed in 1% osmium, dehydrated in an ethanol series, critical point dried, and coated with 10 nm of Au/Pd wire. Specimens were examined with an ETEC Autoscan scanning electron microscope.

Results

Physical and Chemical

Physical and chemical features were relatively consistent over the sampling period. Midday stream temperatures ranged from 21 to 23°C with a mean of 21.8°C. pH ranged from 7.2 to 7.5 and alkalinity from 150 to 170 mg ($CaCO_3$)/L. Light input was also fairly uniform over the sampling dates with a mean light input for 10 min intervals over each incubation period of 339 *W*/s ($\pm SE$ = 16 *W*/s). During the period from 5 July to 13 Sept., 1986, there were only 4 d when rainfall exceeded 2 mm: 2.5 mm on 13 July, 2.2 mm on 27 July, 4.6 mm on 27 Aug, and 4.8 mm on 11 Sept. In spite of the dry conditions, water level did not appear to decrease over the study site, probably due to flow into the stream from springs. Daily air temperatures varied little over the study period: mean daily temperature was 28°C in July, 26°C in Aug., and 25°C in Sept.

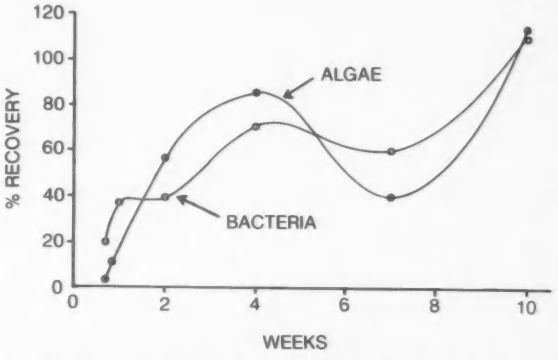

FIG. 2. Relative bacterial and algal activity or recovery calculated from NR productivity divided by OR productivity.

Algal Productivity and Biovolume

Primary productivity on OR varied from 149 (week 10) to 338 mg $C \cdot m^{-2} \cdot d^{-1}$ (week 2), with a mean (n = 7) of 224 mg $C \cdot m^{-2} \cdot d^{-1}$ ($\pm$SE = 26 mg $C \cdot m^{-2} \cdot d^{-1}$) (Fig. 1). New rock primary productivity increased from 7 mg $C \cdot m^{-2} \cdot d^{-1}$ on day 5, to a high of 190 mg$C \cdot m^{-2} \cdot d^{-1}$ by the second week. Both NR and OR primary productivity decreased after week 2. However, a better way of evaluating the recovery of NR communities over time is to view new rock activity as a proportion of OR activity on each sampling date, thereby taking into account seasonal changes (Fig. 2). Viewed this way, recovery of NR community primary productivity steadily increased from nearly 60% at week 2 to 90% at week 4 with a slight decrease around week 7. This decrease coincided with a period when the initial, adnate diatom community had been covered and the subsequent green algal community was still in a developing phase (see below). It was not until the 10th week of sampling that primary productivity of NR communities was equal to or greater than OR communities. By this time, the daily areal production rates of NR communities were actually higher than OR communities (approximately 114% of OR communities) (Fig. 2). Total carbon input from OR for the 5 July–13 Sept. period determined from average daily input multiplied by the interval between sampling dates was 15.67 g C/m^2. Input from NR primary production was 9.21 g C/m^2.

Three major algal taxa were represented on bedrock in Little Schultz Creek: Bacilliarophyceae (diatoms), Chlorophyta (green algae), and Cyanophyta (blue-green algae). The green and blue-green algae were dominated by filamentous forms with prostrate basal portions. The predominant green alga on OR was *Stigeoclonium* sp. and the predominant blue-green alga was *Amphithrix janthina* (Mont.) Bornet & Flahault. Diatoms were dominated by *Gomphonema clevii* Fricke., although *Cocconeis placentula* Ehrenberg was also present along with several species of *Achnanthes* (including *A. lanceolata* (Breb.) Grunow, *A. minutissima* Kütz,and *A. linearis* (W. Smith) Grunow).

After 5 d, exposed bedrock (NR) showed no signs of colonization by algae in electron micrographs (Fig. 3A). However, algal counts using light microscopy indicated a small biovolume, 84% of which were diatoms. At the end of week 1, the biovolume had nearly tripled and micrographs showed a few diatoms (Fig. 3B). At the end of week 2, a monolayer of adnate diatoms (*Cocconeis* and *Achnanthes* spp.) covered exposed rock surfaces (Fig. 3C, 3D), and biovolume had increased 10-fold

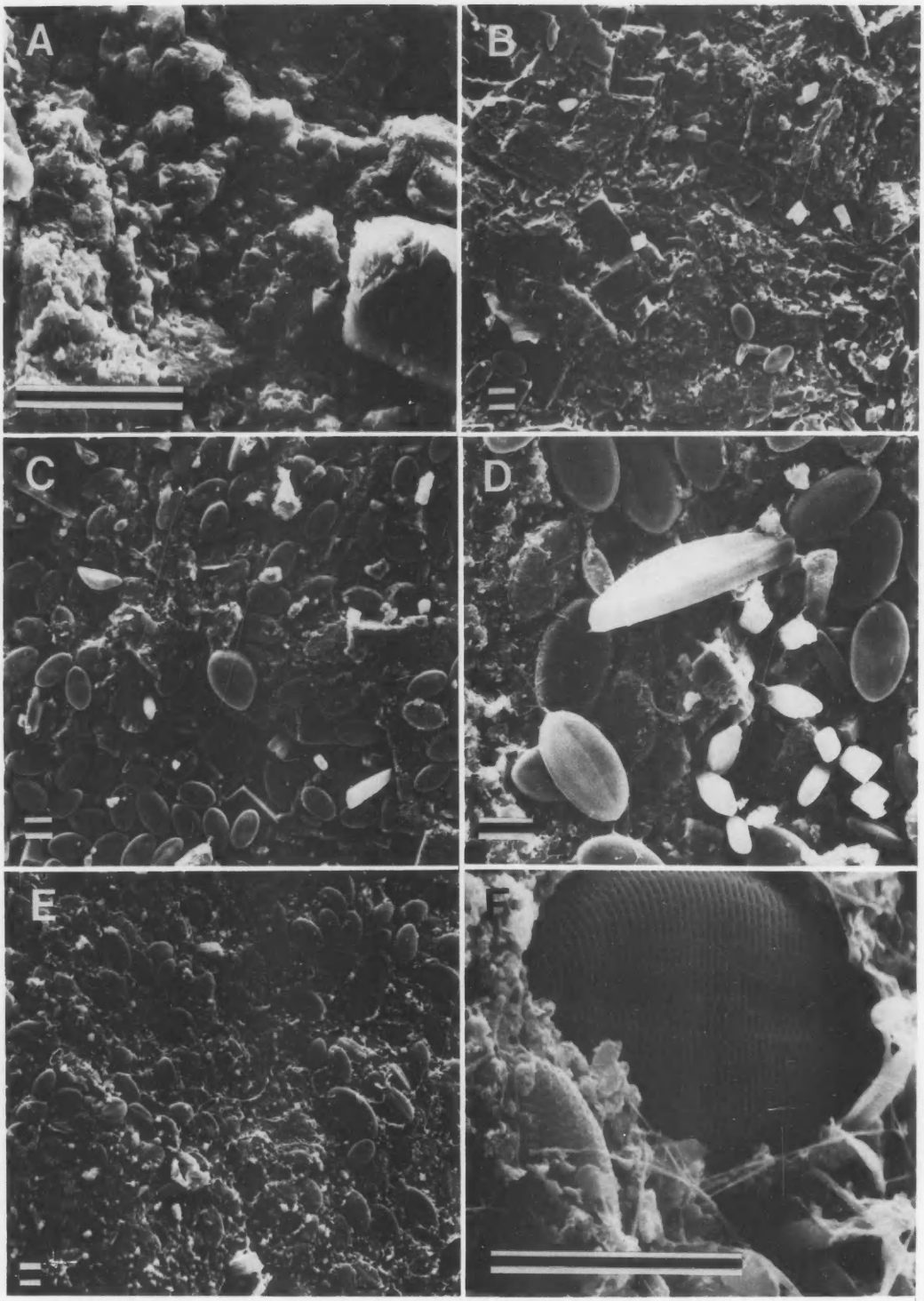

FIG. 3. Scanning electron micrographs of rock surfaces in Little Schultz Creek. A. Recolonized rock (NR), 5 d; B. NR, 1 wk; C. NR, 2 wk; D. NR, 2 wk; E. NR, 4 wk; F. NR, 4 wk; bars = 10 μm.

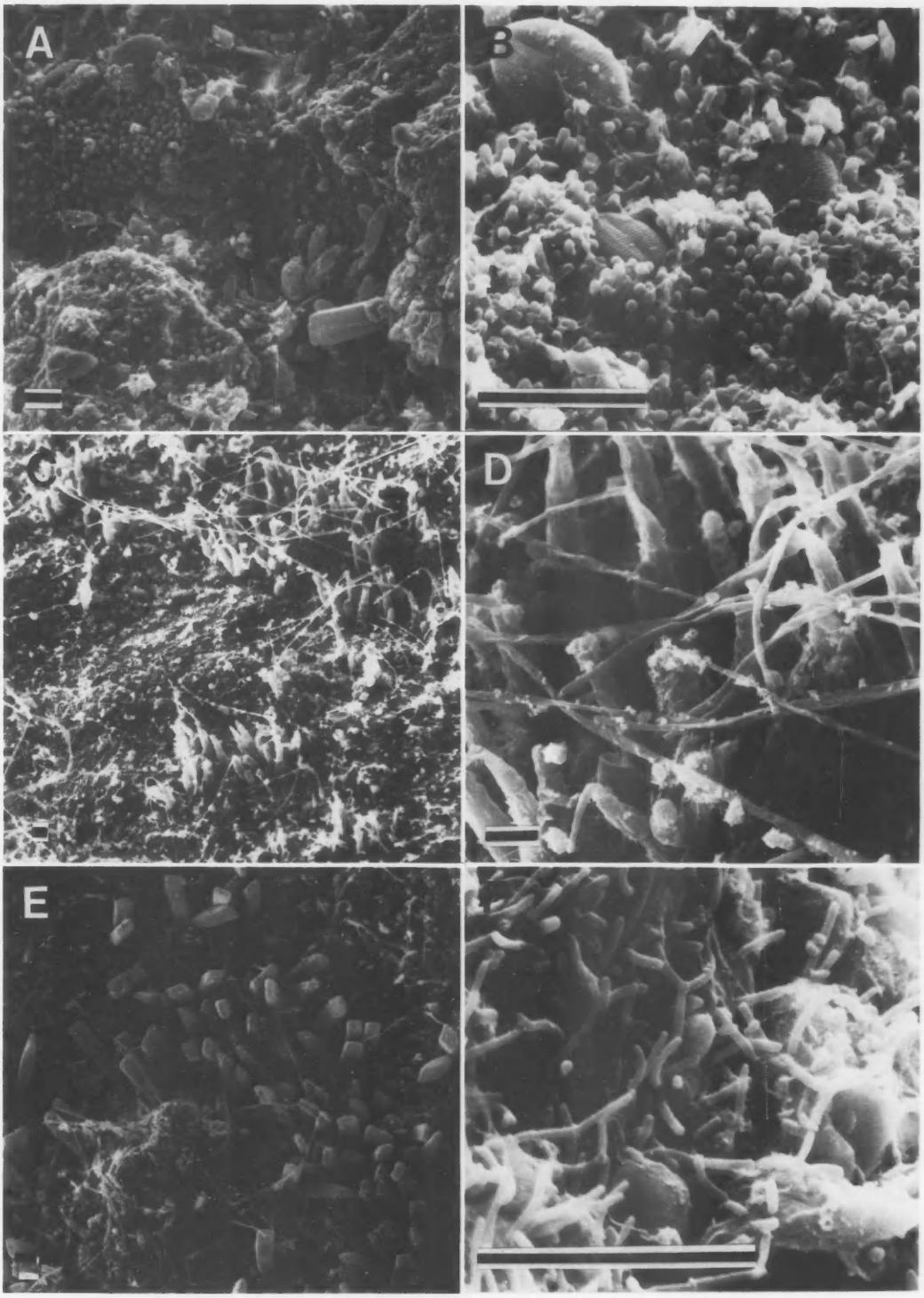

FIG. 4. Scanning electron micrographs of rock surfaces in Little Schultz Creek. A. Recolonized rock (NR), 7 wk; B. NR, 7 wk; C. NR, 10 wk; D. NR, 10 wk; E. Undisturbed rock (OR), 1 Aug. 1986; F. OR, 1 Aug. 1986; bars = 10 μm.

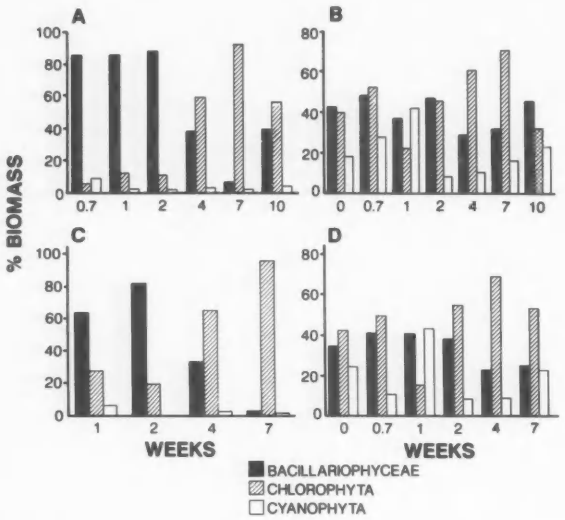

FIG. 5. Relative abundance (biomass of a taxon/total algal biomass) and relative activity (biomass of active cells/total active algal biomass) of algae. A. Recolonized rock (NR) relative abundance; B. Undisturbed rock (OR) relative abundance; C. NR relative activity: D. OR relative activity.

since day 5. Also, the spaces between diatom cells were beginning to fill with mucilage and bacteria (Fig. 3D). Initial colonization was by small diatoms (*Achnanthes* spp.) and stalked *Gomphonema* (relative abundance 30 and 32% respectively) followed, in week 2, by the adnate diatom *Cocconeis* (40% relative abundance).

By week 4, nearly all of the spaces between algal cells were filled, and the mucilaginous complex had begun to overgrow and bury the diatoms (Fig. 3E, 3F). Week 7 was marked by a large increase in the biovolume of green algae, due to the appearance of an over-growth of small basal cells (Fig. 4A, 4B). These cells appeared to be germinalings of filamentous green algae (mainly *Stigeoclonium* sp.). Basal cells had matured to filaments by week 10 (Fig. 4C, 4D). Overall, the NR community showed a predominance of diatoms during the first 2 weeks of the experiment and a subsequent predominance of green algae (Fig. 4A). Blue-green algae were a minor component of the NR epilithic community throughout the study.

Diatoms were codominant with green algae (*Stigeoclonium* sp.) on OR in the early portion of the experiment. The stalked *Gomphonema clevii* was the predominant diatom throughout the experimental period on OR (Fig. 4E, 4F). In contrast to NR communities, OR had substantial green algal biovolume throughout the study with green algae contributing 40% or greater biovolume on 5 of the 7 sampling dates. By week 7, green algae accounted for 92% of the total algal biovolume on NR. Blue-green algae were also relatively more important on OR than on NR throughout the study (Fig. 5B). The relative importance of the three taxa examined appears to have been more equal on OR than on NR, particularly during the early stages of NR colonization. Old rock algal biovolume was relatively consistent throughout the study and for all dates averaged $6.19 \times 10^7 \mu m^3/cm^2$ ($\pm$ SE = 1.0×10^7).

TABLE 1. Bacterial productivity, numbers, and biomass on recolonized (new) rock and on undisturbed (old) rock. P = Productivity; B = Biomass. B/P = Turnover; old rock mean biovolume = 0.77 μm^3/cell; new rock biovolume = 0.82 μm^3/cell.

		P		B	B/P
Week	Date	mg $C \cdot m^{-2} \cdot d^{-1}$	Bacteria/m^2	mg C/m^2	days
Old Rock					
0.7	10 July	84.85	2.07×10^{11}	35.07	0.41
1.0	13 July	52.60	1.05×10^{11}	17.79	0.34
2.0	19 July	72.73	3.02×10^{11}	51.16	0.70
4.0	1 Aug.	98.38	2.59×10^{11}	43.87	0.45
7.0	24 Aug.	79.18	2.75×10^{11}	46.59	0.59
10.0	13 Sept.	41.23	2.08×10^{11}	35.24	0.85
New Rock					
0.7	10 July	16.90	1.16×10^{10}	2.09	0.12
1.0	13 July	19.42	4.53×10^{10}	8.17	0.42
2.0	19 July	28.78	6.55×10^{10}	11.82	0.41
4.0	1 Aug.	69.58	1.39×10^{11}	25.08	0.36
7.0	24 Aug.	47.50	1.14×10^{11}	20.57	0.43
10.0	13 Sept.	45.35	9.22×10^{11}	16.63	0.37

In general, algal activity as determined by autoradiography, mirrored relative abundance. By week 7, the order of relative activity and abundance of the three major algal taxa was the same on NR as on OR: green algae>diatoms>blue-green algae (Fig. 5). On NR, diatoms were the most active (Fig. 5C) in the early portion of the experimental period (66 and 83% of total activity for weeks 1 and 2), and green algae were most active thereafter (65 and 96% of total activity on weeks 4 and 7, respectively). In general, green algae were much more active on NR (86%) than on OR (46%) and blue-green algae were much less important on NR (2%) than on OR (21%).

Bacterial productivity and biovolume

Bacteria quickly colonized NR surfaces with 1.16×10^{10} cells/m^2 present after 5d (Table 1). Bacterial numbers on NR did not attain the same order of magnitude as OR bacteria until week 4. The highest bacterial productivity (69.6 mg $C \cdot m^{-2} \cdot d^{-1}$) was also reached by week 4, which coincided with the filling in of spaces around the *Cocconeis* monolayer. At week 7, productivity decreased slightly but was considerably higher that in the first 3 wk of the experiment. Relative bacterial activity, that is NR/OR production or recovery, increased rapidly to week 1 (37%), was level with that value at week 2 (39%), and then increased to week 4 (Fig. 2). Ten weeks of colonization were necessary before NR productivity was similar to OR. The NR bacterial biovolume was consistently lower than OR biovolume for the experimental period. Turnover times (days) ranged from 0.34 to 0.85 on OR and from 0.12 to 0.43 on NR (Table 1).

Bacterial productivity as a proportion of algal productivity is shown in Fig. 6. The initial proportion for NR was much higher (236%) because algal productivity was very low; as algal productivity increased with colonization, the proportion of bacterial to algal activity on NR decreased rapidly to OR values by week 2. This period coincided with the first major increase in the establishment of the algal community. For the rest of the study, both old and new rock had a similar proportion of bacterial/algal productivity.

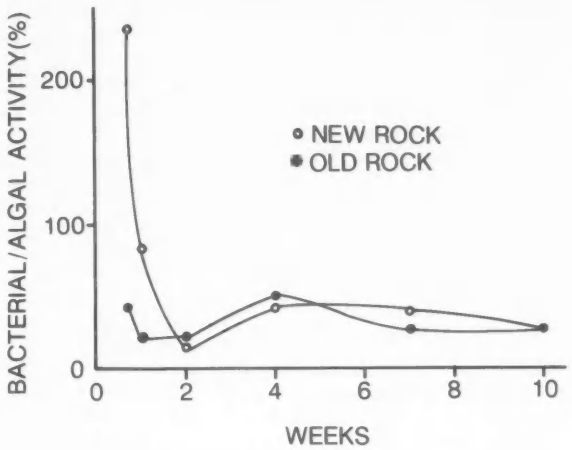

FIG. 6. Bacterial activity as a proportion of algal activity. Old rock (●); New rock (○).

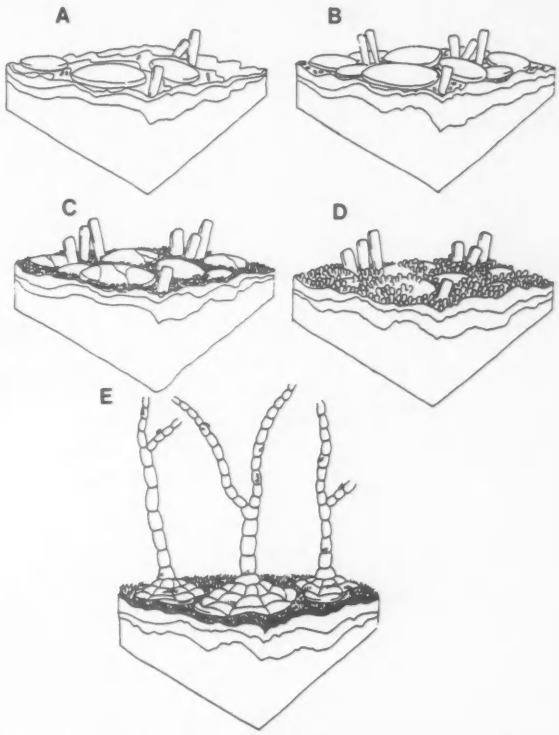

FIG. 7. Illustration of colonization sequence. A. Initial colonization, 0–1 wk; B. Colonization by diatoms, 1–2 wk; C. Bacterial colonization between diatoms, 3 wk; D. Transition period, 7 wk; E. Final community composition, 10 wk.

Discussion

The similarity in the general pattern of per cent recovery for bacterial and algal communities on NR compared to OR tends to imply a strong interaction between the bacterial and algal components of the epilithon. Our conceptualization of the developmental sequence of the epilithon is illustrated in Fig. 7: Fig. 7A, initial bacterial colonization (weeks 0–1); Fig. 7B, algal colonization by adnate diatoms with increasing algal activity (weeks 1–2); Fig. 7C, development of bacteria and mucilage in spaces created by diatoms with steadily increasing activity of both algae and bacteria (week 4); Fig. 7D, transition period with a shift in algal composition from diatoms to filamentous forms (week 7), marked by a decrease in relative algal and bacterial activity compared to OR communities; Fig. 7E, increased recovery to levels at or above those of undisturbed rock with the development of a filamentous algal component of the community (week 10).

One interpretation of the results in this study is that the development of the bacterial component was influenced by the specific growth forms and activity of the algal community and vice versa. We suggest that the successional development of the epilithic community was characterized by a series of interactions in which one component was responding to changes in the growth of the other. For example, the adnate diatoms, *Cocconeis* and *Achnanthes*, were the first algal colonizers; once a diatom monolayer was established, further growth and attachment of these algae were likely prevented by a limited area for attachment. However, bacterial colonization between cells of the diatom monolayer may be facilitated by both the physical spaces (possibly used as a refuge from current velocity and/or grazing) provided by the algae as well as excretion of photosynthetically derived nutrients. The subsequent accumulation of bacteria and mucilage during this phase appeared to bury the diatoms, which may have provided the necessary conditions for the establishment of filamentous algal forms. The development of the later colonizers may be dependent on the establishment of an underlayer of senescent and decomposing algae/bacteria, which provide nutrients for the germination and growth of the spores of the filamentous algae. Additional experimental studies need to be done to confirm the interaction and to test the hypothetical mechanisms of interaction; for example, by darkening one set of substrata and comparing the developing bacterial community in terms of activity and composition with and without the algal component.

The low percentage of active algal cells revealed by autoradiography in this study is in contrast to similar studies of phytoplankton communities. Although many bacteria are present in a dormant state in the environment (Stevenson 1978), no one has examined the relative inactivity of algal cells, assuming that when light is present most cells are active. Contrary to our findings (only 36% of the algal biomass was active), Ellis and Stanford (1982) reported a high percentage (often 100%) of activity of phytoplankton. However, the quantity of activity/cell varied greatly. In planktonic systems one might expect older, senescent cells to be lost to sedimentation; whereas, in attached systems dead or dying cells may be retained longer in combination with viable cells on surfaces. Therefore cells which appear to be viable (i.e., with chloroplasts) when viewed with light microscopy, may actually be relatively inactive photosynthetically. Microenvironmental factors, such as shading by the vertical development of the epilithon and/or microtopographic features of the rock surface, may also contribute to the low percentage of algal activity.

Attached bacteria may utilize dissolved organic carbon (DOC) in the water column or organic compounds produced and excreted by other attached organisms, e.g., algae, as a

TABLE 2. Values for bacterial production as a proportion of algal production, and for bacterial turnover for July and/or August.

Bacteria/ Algae	Turnover (days)	Habitat	Reference
40–70%	0.3–0.6	Eutrophic lake	Bell et al. (1983)
25–30%	—	Lake Mendota	Pedros-Alio and Brock (1982)
8.7–40%	0.3–3.4	Stream sediments	Bott and Kaplan (1985)
23%	—	Howe Sound	Albright and McCrae (1987)
28%	—	Little Crooked Lake	Lovell and Konopka (1985)
44%	—	Crooked Lake	Lovell and Konopka (1985)
15–25%	—	Coastal waters	Fuhrman and Azam (1980)
—	2.0–11.3	Ogeechee River	Edwards and Meyer (1986)
—	1.4	Reservoir	Chrzanowski and Hubbard (1988)
15–50%	0.12–0.85	Stream bedrock	This study

source of carbon and energy (Rounick and Winterbourn 1983). If the production of attached bacteria and algae are linked, then one would expect not only to observe parallels in their activity over time, but also to find that bacterial activity as a proportion of algal activity would be fairly high, or at least relatively constant over time. Reported values for bacterial production/algal production (Table 2) range from 8.7% in stream sediments (Bott and Kaplan 1985) to 70% in a eutrophic lake (Bell et al. 1983). In this study OR bacterial production was well within the reported values as it ranged from 15 to 50% of algal production (Table 2). The parallels between bacterial and algal activity with the structural establishment of the community implies that there is a coupling of these two trophic levels in epilithic communities.

In general the structural sequence from adnate diatoms to a more complex structure with stalked diatoms and filamentous algae seen in this study was similar to the natural successional or colonization order that has been reported in other studies (Hoagland et al. 1982; Korte and Blinn 1983; Steinman and McIntire 1986). The NR successional sequence from diatoms to green algae paralleled the similar natural seasonal shift seen on OR. Despite these similarities, there were also some potentially important differences between NR and OR algal communities. Overall NR relative cell-specific activity was higher (46%) than that on OR (36%) (see Fig. 5).

New rock microbial communities developed on rock substrata which may have lacked a mature alteration zone; that is, a region on the rock surface where weathering reactions have occurred and which has a chemical composition different from underlying bulk rock. We have also observed endolithic blue-green algal growth in that region, which we believe acts as an inoculum for overlying communities (Stock 1988). The removal of the upper rock surface and associated blue-green algae prior to development of NR communities may have contributed to the lesser importance of blue-green algae in that community compared to OR communities.

Even though the composition of the communities on NR and OR differed at the end of the experiment, they were similar in physiognomic structure (with filamentous forms more prevalent than adnate forms), and NR bacterial and algal activities were actually higher than OR. The higher level of total epilithic activity on recolonized rock after 10 weeks may indicate that some level of disturbance can maintain more vigorous growth and productivity than when there is less disturbance of the epilithon. This is analogous to the stimulatory effect of intermediate grazing pressure by aquatic invertebrates in streams (Gregory 1983). However, despite intense grazing pressure by snails on NR and OR communities (Stock et al. 1987; Richardson et al. 1988), neither community was maintained in an early seral stage, and the NR community progressed past the diatom monolayer stage by week 4.

One aspect of benthic algal communities which has frequently been overlooked in laboratory studies examining algal/grazer interactions has been the potential contribution of the associated bacterial productivity to invertebrate growth. Although there have been many studies of heterotrophic bacterial production using ^{3}H-TdR in lakes, marine systems, and sediments, only one study of bacterial activity on rock surfaces has been reported (Palumbo et al. 1987). In a circumneutral stream they reported 2.5 × fewer cells (8×10^{10}/m^2 vs 2×10^{11}) than in this study and approximately 18 times less activity (12 nmole TdR·m^{-2}·d^{-1} vs an OR mean of 211 nmole TdR·m^{-2}·d^{-1}). However, their study was conducted in October at much colder temperatures than in Little Schultz Creek in summer, which may account for their much lower bacterial activity and numbers. Our bacterial numbers were similar to those found in a stream by Haack and McFeters (1982a), who reported 1.9×10^{11} to 4.4×10^{12}cells/m^2 from July through October. Bacteria in Little Schultz Creek were quite active with turnover times consistently less than one day (range = 0.12–0.85). Summer (July and August) turnover rates from some freshwater systems vary from 0.3 to 11.3 d (Table 2). Our OR turnover values are similar to that found in a eutrophic lake (Bell et al. 1983) and stream sediments (Bott and Kaplan 1985). One would expect a newly colonized community would be more active than an older community, and this was the case here, with NR bacteria having faster turnover than OR bacteria. For example, at the end of the experiment even though OR and NR bacterial productivities on an areal basis were nearly the same (OR = 41 mg C·m^{-2}·d^{-1}; NR = 45 mg C·m^{-2}·d^{-1}), biomass on NR was much lower; therefore, these bacteria were much more active per unit biomass. However, colonization showed no apparent trend in turnover rates as the community became established.

Both algal and bacterial production contribute to epilithic biomass and, therefore, are two components of the community that are available for consumption by macroinvertebrate grazers. Total epilithic production (bacterial + algal) during the period of this study was 20 g C/m^2 with approximatey 78% from algae and 22% from bacteria. Although we did not address directly the importance of epilithic production to macroinvertebrate production in Little Schultz Creek, using snail production data from Richardson et al. (1988) (0.6 g C/m^2 from July–mid–Sept. 1985) and total epilithic production from this study (20 g C/m^2), we calculated that 3% of the latter was incorporated into snail biomass over a 2.5-mo summer period. Even if assimilation values as low as 10% are used, there was more than enough epilithic production to support grazing snails in this system. While data are not available to determine the proportion of total secondary production that is due to autochthonous organic matter, epilithic production was clearly of major importance to snail secondary production.

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Larval Fish Age, Growth, and Body Shrinkage: Information Available from Otoliths

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External and internal examination of Atlantic cod (*Gadus morhua*) otoliths for macrostructure and microstructure, by light and scanning electron microscopy, indicated daily rhythmic patterns. The first daily increment developed the day after hatching. Sagittae changed shape from spherical to oblong at 20d and to crenulated at 50–60d old. Cod were reared at three temperatures (6,8 and 10°C), to provide a range of growth and developmental rates. Distinctive marks formed at yolk-sac absorption, initiation of feeding and settlement.It was possible to determine age and growth rate from otolith analyses. The relationship between otolith length and fish size was independent of growth rate; it followed a quadratic function for the smaller individuals (<6.5 mm), and it was linear in individuals greater than 25 mm. Larval fish shrank considerably at death. The magnitude of shrinkage was dependent on larval length, and the elapsed time between death and fixation. Immediate fixation in ethanol resulted in minimal shrinkage. The relationship between fish length and otolith diameter may be used to correct for shrinkage associated with collection and death.

L'examen interne et externe de la macro et de la microstructure d'otolithes de la morue franche (*Gadus morhua*), par microscopies optique et électronique à balayage, a révélé la présence de rythmes quotidiens. La première augmentation quotidienne est apparue le jour après l'éclosion. Les sagittas, sphériques, sont devenues oblongues à 20 jours et crénelées à 50–60 d. Trois groupes de morues ont été élevés à des températures différentes (6, 8 et 10°C), ce qui donne diverses vitesses de croissance et de développement. Des marques caractéristiques sont apparues lors de la résorption du sac vitellin, du début de l'alimentation et de la descente vers le fond. L'analyse des otolithes a permis de déterminer l'âge et le taux de croissance des larves. Le rapport existant entre la longueur des otolithes et la taille des poissons était indépendant du taux de croissance; ce rapport avait la forme d'une fonction quadratique pour les poissons plus petits (<6,5 mm) et était linéaire pour les poissons de taille supérieure à 25 mm. Les larves de poissons ont rétréci de manière considérable à leur mort. L'importance du rétrécissement dépendait de la longueur de la larve, ainsi que du temps écoulé entre la mort et la fixation. La fixation immédiate à l'éthanol a permis d'obtenir un rétrécissement minimum. Le rapport existant entre la longueur des poissons et le diamètre des otolithes peut servir à corriger le rétrécissement lié à la récolte et à la mort.

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The discovery of microincrements in otoliths — analogous to annual increments, but formed on a daily basis — has permitted the ageing of larval fish. Daily growth increments are displayed in the structures of many animals (Neville 1967), and their formation in fish otoliths was first postulated by Pannella (1971, 1974). Subsequent work by Brothers et al. (1976) verified daily increment deposition for several fish species reared from eggs in the laboratory. Since then numerous studies have dealt with daily increments in reared and wild fish (e.g. Campana and Neilson 1985; Jones 1986a).

Although daily increments occur in most species studied, validation of the frequency of increment deposition is a necessary prerequisite for age and growth studies that use otolith microstructure. Three methods of validation are: (1) the use of known-age fish; (2) sequential sampling of a population in which neither age-selective migration nor age-selective mortality occurs; and (3) the introduction of dated marks onto the otoliths. The first method is the preferred one, especially in the study of larval fishes, since increment formation is initiated at different ages depending on the fish species (Neilson and Geen 1982, 1985; Brothers et al. 1976; Radtke and Dean 1982). In the present study, daily increment deposition was validated in Atlantic cod (*Gadus morhua*) using laboratory-raised larvae and juveniles. Precise ageing provides accurate estimates of growth rates, making other larval fish population parameters more reliable.

Besides permitting age determination, otoliths can provide other information about larval fish biology and ecology. Otoliths have been used to determine temperature histories (Radtke 1989; Radtke et al. 1989) and migration patterns (Radtke et al. 1988; Radtke and Morales-Nin 1989). In this study otolith microincrements are analyzed for life-history changes in larval and juvenile cod, and increment width is related to growth rates.

Numerous authors (e.g. Blaxter 1971; Theilacker 1980; Hay 1981; Leak 1986) have demonstrated a difference in measurements between laboratory and field-collected specimens due to shrinkage. Larvae, in particular, are susceptible to shrinkage. Although most studies have focused on the effect of preservatives on fish size (e.g. Blaxter 1971; Schnack and Rosenthal 1978; Tucker and Chester 1984), death itself may be the major cause of shrinkage (Theilacker 1980; 1986). In the present study postmortem shrinkage in cod is analyzed in individuals ranging

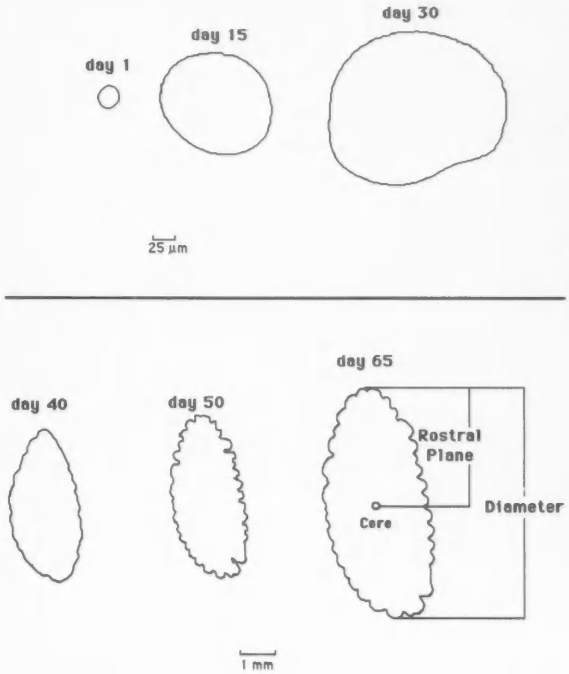

FIG. 1. Changes in shape of the sagitta of the Atlantic cod, *Gadus morhua*, as related to developmental time. Otoliths from newly hatched larvae through juvenile fish elongate along the rostrum followed by dentation of the edges. Otolith diameters were measured for the spherical otoliths. Lengths of the elongated sagittal otoliths of cod were taken along the longest plane within the otolith as shown, and incremental widths were obtained from the outermost increment in the rostral plane.

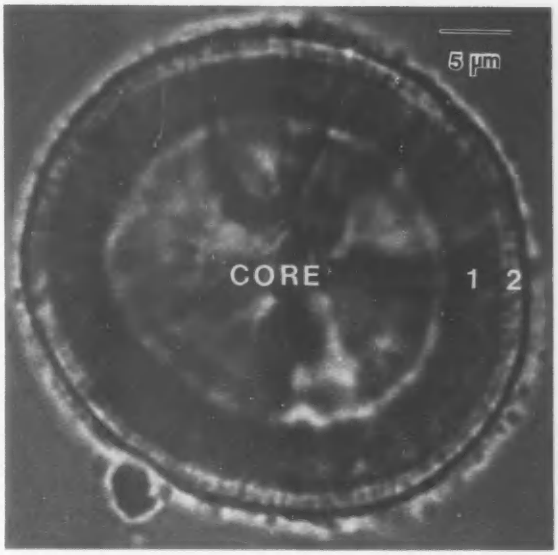

FIG. 2. A sagittal otolith from a 3-d-old cod larva displaying alternating light and dark zones, as viewed by light microscopy. The core of the otolith is not enumerated as an increment, since it was formed during embryological development.

from hatching through development into juvenile. A relationship between fish length and otolith size was determined and the use of otolith size to account for possible shrinkage is evaluated.

I used cod for these investigations because of its wide North Atlantic distribution (Dannevig 1933; Pinhorn 1969), its prominence in North Atlantic marine ecosystems, and worldwide interest in cod biology. Furthermore, cod have pelagic larvae which are subjected to a wide range of physical and nutritional conditions over a short time. The use of otoliths in the study of the effects of growth on survival and recruitment increases the understanding of life-history patterns in cod larvae and juveniles, and makes it possible to address important questions on how fish larvae function in the ocean.

Materials and Methods

Cod were reared at the Flødevigen Biological Station, Arendal, Norway. Fertilized eggs were obtained and incubated with a photoperiod of 12 h of light and 12 h of darkness. Only larvae which hatched within 24 h of each other were used. Data recorded included: development with respect to yolk absorption and gut differentiation, body growth, and total length. The eggs were incubated at water temperatures of 6, 8 and 10°C. Experiments lasted 4–6 mo.

Hatching times were noted and the fish were then fed ad libitum with plankton collected in the adjacent fjord. At each sampling, 10 fish from each temperature group were taken for otolith and growth measurements. Another 10 fish were sampled to study postmortem shrinkage, and 10 fish were preserved in 96% ethanol while still alive. Samples were taken daily for the first 15 d and weekly thereafter.

In the shrinkage experiments, the fish were removed from the incubation tanks while still alive, placed in a depression slide, and measured with an ocular micrometer to the nearest 0.01 mm. The fish were then allowed to die, and the time of death (based on cessation of heart movement) was recorded. All sacrificed fish were measured 15 min after death by exposure to air or alcohol. Otoliths were prepared for light microscopy, the sagittae were measured, and these measurements were correlated with the total length of the fish before death.

Sagittae were too large for whole mounts in juveniles, so they were ground from the proximal and distal sides using a size-graded series of grinding compounds. Transverse sections at the focal level appeared to give the most complete picture of the increments found in the otolith and these were chosen for routine use. Such sections are described by Steffensen (1980). In preparation for light microscopy, otoliths were mounted on glass slides using a mounting medium having a similar refractive index as that of glass, and covered with a glass cover slip.

In preparation for internal structural analyses, otoliths from larvae and juveniles were mounted whole on SEM viewing stubs using 5-min epoxy resin. The otoliths were ground with 600 grit sand paper and polished with 0.3 µm alumina polish. The polished surfaces were then decalcified with 7% EDTA at pH 8 for 5–15 min. Following decalcification, the samples were coated with gold and observed in a SEM.

Larval otoliths and otolith increments were measured under a light microscope using an ocular micrometer. Larval otoliths

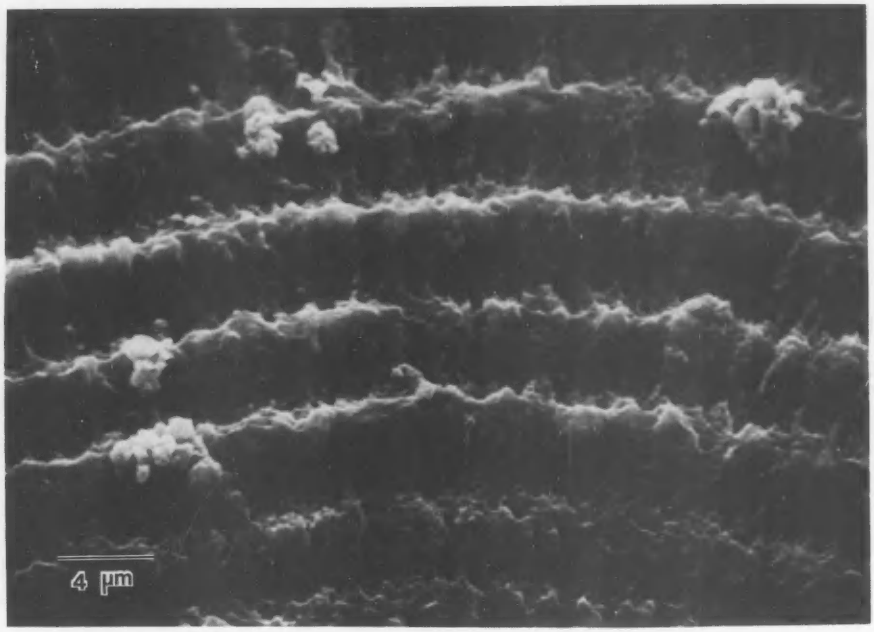

FIG. 3. Scanning electron micrograph of the microstructural components of cod sagitta demonstrating protein deposition which produced distinct increments. These increments were deposited daily.

FIG. 4. Scanning electron micrograph of a sagitta from a 37-d-old cod larva with increments (arrow) which began formation at the time of hatching. Faint increments are observed inside the check.

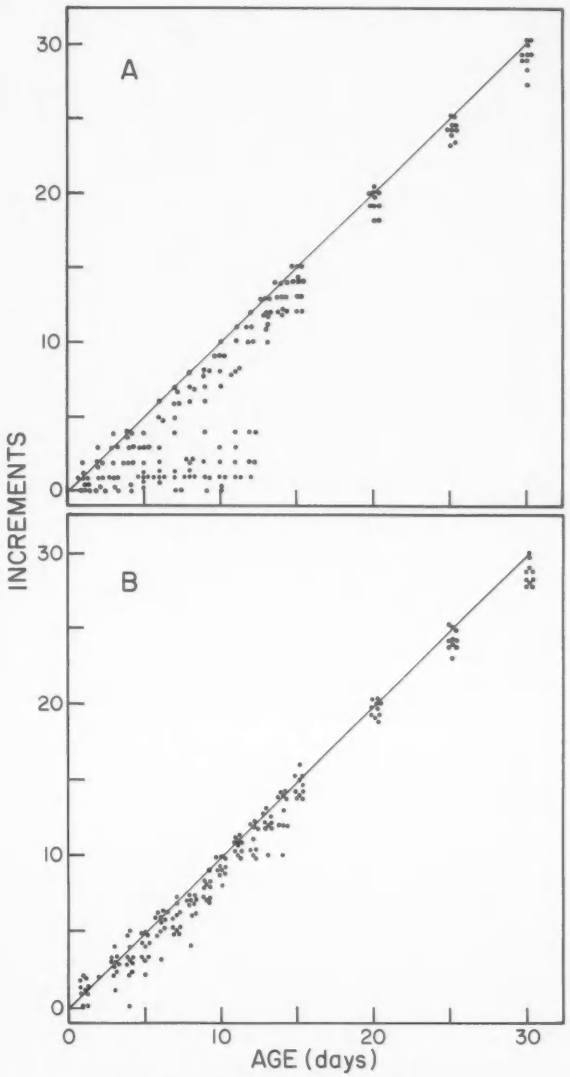

FIG. 5. The relationship between otolith (sagitta) increments and age (days) in cod larvae. (A) Larvae from culture 1 were sampled at random and all individuals were viewed. A large mortality took place at day 12; (B) Larvae from culture 2. Only actively swimming individuals with food in their guts were examined for daily increments.

are nearly spherical and thus appear circular. A simple measurement of the longest length was found to be acceptable for describing their growth. When the sagitta began to show signs of differentiation into other planes, the otoliths were measured along their longest anterior/posterior axis. A diagram of measurements taken is shown in Fig. 1. Each increment is composed of a discontinuous and an incremental zone (Tanaka et al. 1981; Radtke and Dean 1982), which together were counted as one increment. In some fish one or two faint pre-hatching increments were observed by SEM in the core area. These were not counted, and neither was the core counted. The increment appearing closest to the core was usually more prominent than the outer increments and was chosen as the starting point for counting and measuring increment widths. To count increments, the otoliths were presented to the author in a random and undisclosed sequence with respect to age. Three independent readings were performed by the author on each otolith. The counts did not vary significantly and the median was accepted. Approximately 5% of the otoliths were discarded due to abnormal shape, breakage during handling, or difficulty in reading. Both the left and right otoliths of the larval fish were counted, but no significant differences were observed. Thus, the mean for the two otoliths were used to estimate age.

In addition, one cohort of hatchlings was not fed and kept at 6°C. After 10 d all larvae were still robust and demonstrated healthy behavior. These fish were then sacrificed and examined for increment formation

Only the right otolith for each juvenile cod (20 d or older) was counted and measured. Otolith shapes and structural properties were analyzed with a computer digitizer. Counts of composite zones, consisting of one incremental and one discontinuous zone, were made. All sections on juvenile fish were found to be readable. Increment width measurements were made on the outermost increments of sagittae examined in the rostral plane. All counts and measurements were made with a light microscope. Scanning electron microscope (SEM) measurements and counts were done on a subsample, usually one otolith per group of fish, to compare to the light microscope results.

Results

The sagittae and lapilli of developing cod were the first tissues to calcify, as determined by polarized light examination. Calcification of these otoliths occurred just before eye pigmentation during embryogenesis. The asterisci were not formed at the time of hatching, and did not become apparent until the fish reached about 6 mm in length, or about 20 d of age. At hatching, the lapilli were slightly larger than the sagittae, but this size difference was reversed within a few days. Diameters averaged 26.8 ± 1.7 μm at hatching for lapilli, and sagittae measured 4.0 μm smaller.

From the otolith morphology it was possible to distinguish three stages in sagittal growth (Fig. 1). The first stage (spherical stage) occurred in larvae of approximately 20 d or younger, and was characterized by nearly spherical sagittae. Thereafter, otolith shape began to elongate and a sulcus became apparent (oblong stage). At 50–60 d of age a third phase in otolith growth (crenulated stage) could be distinguished, and sagittae became distinctly crenulated.

Spherical Stage

There were distinct increments of microstructure originating at the core region. When otoliths were examined under transmitted light, the increments appeared as alternate light and dark areas (Fig. 2). Under the SEM microincrements could be enumerated and measured from the rugose surface of prepared otoliths (Fig. 3). The fertilized eggs reared at 10,8, and 6°C hatched after 10, 12, and 14 d of incubation, respectively. In a majority of larval otoliths, a prominent increment was found close to the core (Fig. 4), corresponding to hatching. Outside the core the matrix was arranged in concentric, undulating bundles with growth increments beginning to form the day after hatching.

Increments examined using light microscopy appeared to form on a daily basis. Otoliths from laboratory-reared larvae had less distinct increments than those from wild larvae. When

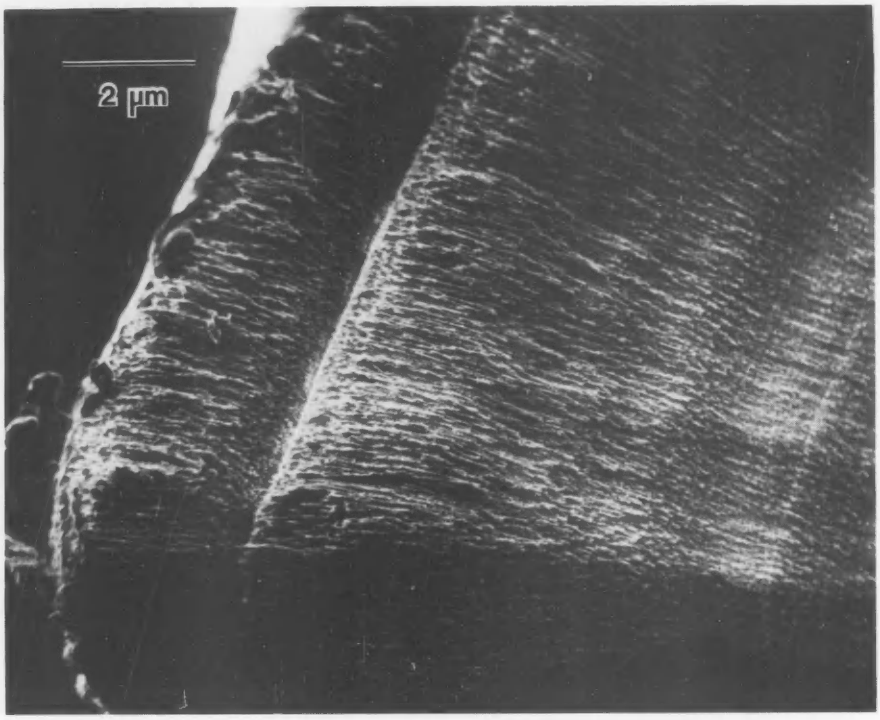

FIG. 6. Scanning electron micrograph of a sagitta from a 7-d-old cod showing a distinct incremental check corresponding to the time of yolk-sac absorption and first feeding. The ridges are the protein matrices of each increment after decalcification.

increments in the sagittae of a random sample of larval cod were viewed, their frequency approximated one per day (Fig. 5A), except for the first 12 d after hatching. For actively swimming and feeding larvae the relationship was closer to one increment per day (Fig. 5B). The state of health or physical condition of the larvae examined from culture experiments appeared to affect increment counts. In moribund larvae, increments seemed to cease forming and this could confound the interpretation of counts if these larvae were examined mixed with healthy ones. The present study suggests that with experience, non-competent larvae could be distinguished solely on the appearance of their otoliths.

The yolk-sac phase ranged from 4 d at 10°C to 12 d at 6°C. Most otoliths demonstrated a definite transition zone at the end of yolk-sac absorption (Fig. 6). After yolk-sac absorption, growth was slow for 7–20 d at all three temperatures. The highest mortality occurred during this phase. This transition to active feeding may not be successful in many individuals hence the high mortality rate. The onset of feeding in larvae produced heavier, more distinct increments. Faint daily increments, which were often difficult to interpret, were observed in otoliths of larvae that had been starved for up to 10 d. They could easily be misinterpreted if not observed with the necessary caution.

The sagitta and lapillus grew slowly during the first 15–20 d, and otolith growth was related to larval growth (Fig. 7B and 8A). Increment widths were narrow during this period of slow growth, and the widest increments were observed at the highest temperature. Sagitta diameter was found to be a good predictor for larval length, and during the early growth phase, the relationship between the size of the otolith and the size of the fish was best fitted by a quadratic curve (Fig. 8A).

Oblong and Crenulated stages

Cod sagittae changed as they grew, from spherical to elongated to crenulated over a 65-d period (Fig. 1). The proximal surface of each sagitta was oriented toward the central axis of the fish. The convex surface was demarcated by a shallow sulcus, the depth of which increased with growth. The sagittae have a rounded rostrum with an undifferentiated antirostrum (nomenclature from Hecht 1978). The margins of the sagittae began dentation at a TL of 50 mm, and there was an increase in dentation with growth. No distinct excisural notches were present. The sagittae were robust, with a shape distinguishable from other gadoid species. These changes in shape are the result of peripheral primordia which make it possible for different shapes to be formed. However, the primary primordia were still present in the otoliths and could be traced from the core area to the edge of the otolith for accurate increment counts. Changes in otolith shape are reflected in the relationship between fish and otolith lengths, as the fish reaches metamorphosis and begins a benthic lifestyle. In addition to the changes in otolith shape, a transitional area was visible at the time of settlement, at 80–90 d of age.

Daily increments were documented through the first 60 d. Some variation in individual growth occurred, but daily incre-

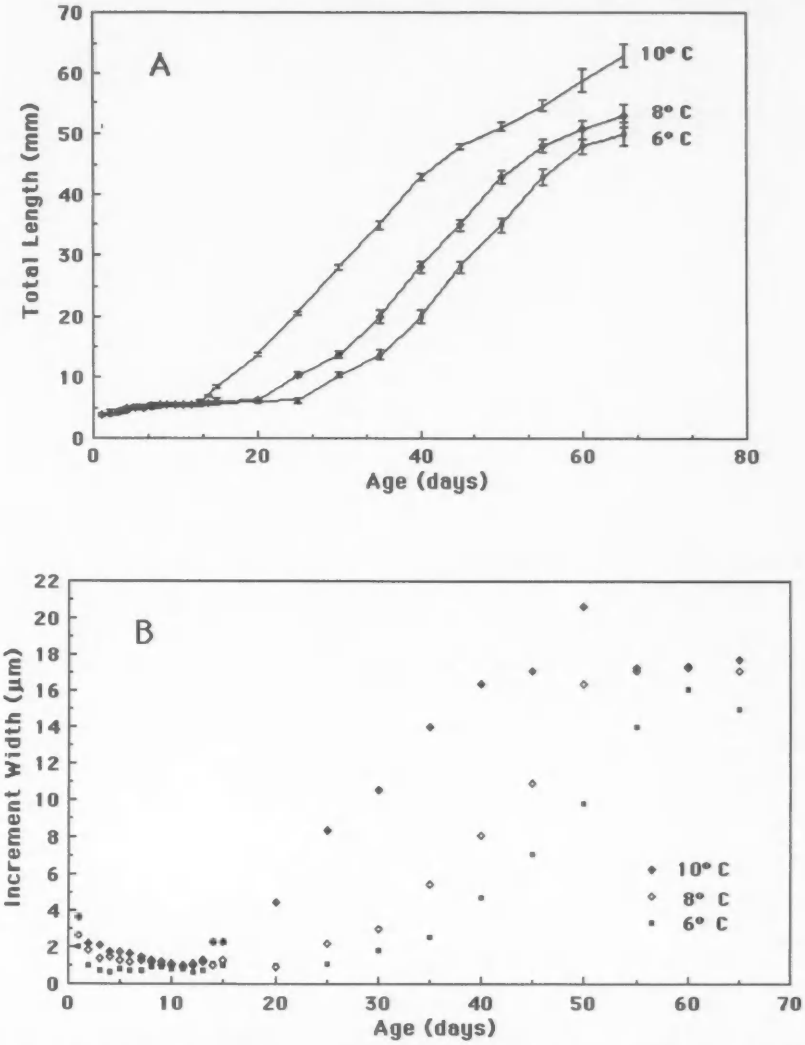

FIG. 7. Temperature related differences in growth and increment width for cod. (A) Changes in total length associated with age in days of cod reared at three different temperatures. Data presented for each point are the means of measurements from 10 fish ± one standard deviation; (B) Width of the outermost increments in cod sagittae as related to age.

ment deposition did not vary significantly. In a comparison between number of increments to age in days, the slope of a straight line fitted to the data was not significantly different from unity ($P > 0.05$). An average of 30 increments were found in the sagittae taken from juveniles with a mean age of 31 d. After the initial slow growth phase, otoliths and juveniles grew very rapidly (Fig. 7), and sagittae increment width increased.

The oblong stage was characterized by nearly linear increase in total fish length at all three temperatures (Fig. 7). The highest growth rates were observed between fish lengths of 6.8 and 47.5 mm at all three temperatures. This period of rapid growth occurred between 13 and 45 d at 10°C, 20 and 55 d at 8°C, and 25 and 60 d at 6°C. Growth rates were 1.22, 1.22, and 1.33 mm/d at 6, 8, and 10°C, respectively. The approximately 10% difference in growth rates among these three temperatures is small compared to the temperature effect on length of the yolk-sac stage and subsequent initiation of feeding.

Total juvenile length was a linear function of otolith diameter for the oblong and crenulated stages (Fig. 8B). This relationship is different from the one observed for the younger larvae (Fig. 8A). Over the entire growth period there were two inflection points (marked by arrows) which differentiate the otolith size–body size relationship into stanzas. At the inflections there appears to be a stalling in otolith growth, and major axis growth may be diverted into accessory primordia. Over the entire growth period the combined data fitted a third degree polynom-

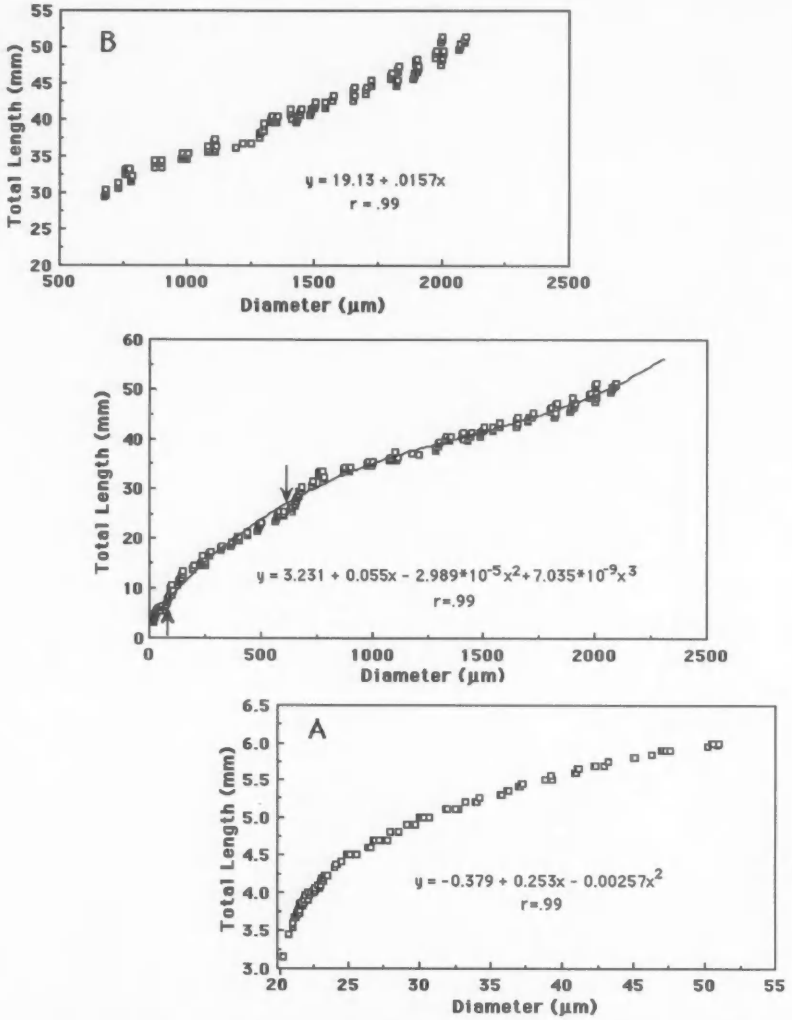

FIG. 8. The relationship between otolith size (diameter) and total length of live larvae in the cod. The figure can be broken into two growth stanzas, A and B, showing growth for the smallest (<6.5 mm) and the larger (>25 mm) larvae, respectively. (A) Demonstrates growth best described by a quadratic equation and shows that there is a rapid increase of growth with a slight leveling of otolith dimensions as the fish grows; (B) Demonstrates that the relationship between total length and otolith diameter is linear during the oblong and crenulated stages.

ial with a correlation coefficient of 0.99 (Fig. 8, middle). Once the relationship between body size and otolith size is established, it may then be used to correct for shrinkage caused by handling. However, caution is necessary at the inflections, where the cubic polynomial is a poor fit.

Body Shrinkage of Cod Larvae

Newly hatched larvae shrank from 30 to 40% within 15 min of death and over 40% overall. Shrinkage appeared to be related to the size of the fish (Fig. 9A and B, Table 1), and no statistical differences were found between fish raised at different temperatures ($P > 0.05$). Shrinkage was greatest in fish between 3.5 and 6 mm in length. Percent shrinkage, y, was related to fish total length, x, by the equation $y = 43.969e^{-0.04x}$ ($r^2 = 0.99$, Table 1). Shrinkage was almost negligible (about 1% for newly hatched larvae, and as low as 0.2% for older fish) in cod that were placed into ethanol directly after death. The shrinkage phenomenon may create problems with plankton net collections when portions of the collection have been dead for 15 min while other portions are still alive upon preservation.

Discussion

Cod growth and development rates can be determined through the analysis of otolith microincrements, which can be counted and measured with a light microscope or a SEM. Light micros-

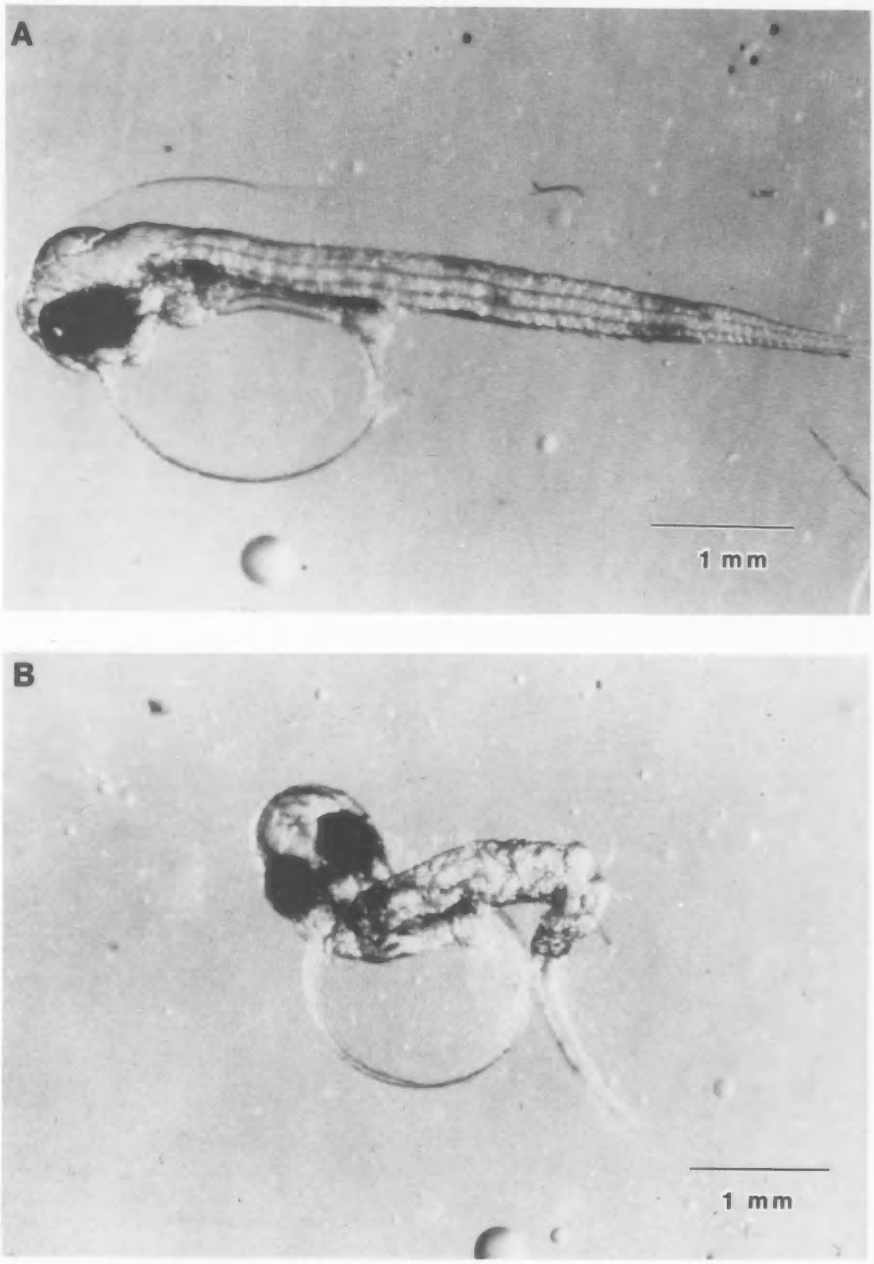

FIG. 9. Shrinkage of young cod larvae associated with death. (A) Young larva which is still alive demonstrates no shrinkage. All lengths taken in the present experiments were from live fish; (B) The same larva 15 min after cessation of heart activity. There is a rapid contraction of the body, and measurements for shrinkage were taken from these larvae once the tails had been extended again.

copy is relatively simple and can be used routinely for large numbers of samples. In this study this technique was used successfully for all routine counts. Under light microscopy, transmitted light is refracted at obtuse angles at the otolith edge due to its curved surface. As a result microincrements may be difficult to distinguish, when faint boundaries occur. When using SEM, visual artifacts do not occur, and increments, which are not easily discerned with a light microscope, can be seen clearly (Campana and Neilson 1985). Otolith increment width measurements are more precise when digitized from SEM photo-

TABLE 1. Shrinkage of the Atlantic cod, *Gadus morhua*, as caused by death processes, and its relationship to fish size.

TL (mm)	% shrinkage	SD
3.618	40.15	2.6
3.784	39.35	3.9
4.140	39.35	4.0
4.402	30.05	4.6
4.710	39.05	3.3
4.776	38.75	3.8
4.916	38.0	2.1
5.182	37.25	3.8
5.322	37.25	3.8
5.488	36.60	2.8
5.430	36.30	4.3
5.444	36.15	3.9
5.472	35.70	4.8
5.570	35.55	5.1
5.612	34.95	5.2
6.084	33.40	6.9
10.0	27.0	7.0
13.75	25.3	3.2
20.0	20.6	5.9
28.0	18.3	3.2
35.0	15.0	3.6
43.25	11.3	3.0
48.0	8.0	3.7
50.0	6.0	5.1

graphs or video-taped images, than when measured with a light microscope. SEM techniques have been used to confirm otolith structure (Dunkelberger et al. 1980; Watabe et al. 1982) and to compare increment counts to those obtained by light microscopy (e.g. Radtke and Dean 1982). However, extensive use of SEM for field surveys is impractical due to the cost and preparation time required. Confirmation of light microscope counts by SEM is highly desirable.

Otoliths form early in cod larvae, but I found no increments before hatching. The lack of increments in the initial stages of otolith formation suggests that the deposition of growth material takes place at a steady rate. Increment formation began at hatching and was continuous thereafter. The formation of growth increments in otoliths is probably due to differential rates of production and/or deposition of growth material, which is reflected in differences in density of otolith deposits. The highest density of organic matrix occurs when deposition is resumed after a resting phase, during the active growth phase, which occurs at dawn (Tanaka et al. 1981). This layer also corresponds to the interlamellar organic matrix as shown by Dunkelberger et al. (1980), Tanaka et al. (1981) and Radtke and Dean (1982). These authors demonstrated that discontinuous zones in otoliths contain more organic matrix than incremental layers.

Three major landmarks were observed in the present work, the core check at hatching, the check marking yolk-sac absorption, and the transition zone at metamorphosis. Bolz and Lough (1983, 1988) observed check rings at hatching and yolk-sac absorption in field-caught Atlantic cod, but could not discern the settling mark. Little is known of the physiological processes responsible for check formation. Thus, it is not clear when checks would be expected to form. A source of error in ageing larval fish is associated with the date of formation of the first increment. The date of first increment formation has been reported from before hatching (as in salmon, Neilson and Geen 1982, 1985; and in *Fundulus heteroclitus*, Radtke and Dean 1982), to the time of first feeding (as in anchovies, Brothers et al. 1976). In the absence of data concerning the age of first increment production, many researchers have assumed that it coincided with an event of biological significance in the life history, such as hatching. Assumption of an incorrect event could be responsible for the poor correspondence between observed and expected increment counts reported in some studies (particularly for young fishes) (Jones 1986).

It appears that duration of the yolk-sac stage and subsequent adjustment period to active feeding are temperature-dependent, as evidenced by differences demonstrated by larvae raised at 6 and 10°C. Yolk-sac absorption in field-captured Atlantic cod on Georges Bank was observed to occur at 2–8 d of age (Bolz and Lough 1983), which suggests that these larvae experienced, on the average, higher temperatures than the larvae in the present study, where the yolk phase lasted from 4 to 12 d at 10 and 6°C, respectively. The present laboratory experiments demonstrated that faster development rates occurred at higher temperatures. However, microincrements were still deposited daily in both larvae and juveniles. This daily deposition occurred at all three temperatures and in both starved and fed animals, in spite of differences in development rates.

Otolith size was found to be a good predictor for fish size, independent of temperature. Increment width increased with temperature in response to faster growth. Interpretation of increment width depends on the stability of the relationship between otolith size and fish size. The formation of accessory primordia in the otoliths of juvenile cod fish may confuse the interpretation of daily increment width, and it is important to measure increment widths using the same field of measurement both within an otolith and between specimens. However, used with caution increment width can be used to determine the history of the daily growth rates in an individual cod fish.

Rapid growth during the first months of life may be critical to survival (Houde 1987). Variation in recruitment may in part be explained by differences in developmental rates, which are affected by both temperature and food levels. Otolith increment widths and the position of check marks are indicative of growth and development rates, and could be used in recruitment studies. Jones (1985) found that differences in growth estimates between herring larvae hatched early in the season, and those hatched later could be demonstrated through use of otolith increments. Differences in overall mortality may be associated with these differences in growth rate (Methot 1983).

Wild cod larvae have an average growth rate of 0.3 mm per day during the first 2.5 mo (Bolz and Lough 1983, 1988), which is much lower than the overall growth rates of 0.7 to 0.9 mm per day observed during the first two months in the present experiment. This is not surprising, since the lab cod fish were fed ad libitum. The growth rates determined in the present study are in agreement with work by Steffensen (1980) on increments in juvenile Baltic cod. The fish in his collections were larger at a given age than those found in the 6°C group, but close in size to those in the 10°C group. For example, in his experiments, a 60 mm fish had only 46 increments. Gjøsaeter and Tilseth (1982), investigating growth rates in a North Sea cod, found slow growth in the small (< 10 mm standard length) larvae which is similar to the results in the present study.

Body Shrinkage of Cod Larvae

Lengths of larval fish are routinely measured during ichthyoplankton surveys to estimate larval age, growth and mortality, and occasionally are examined to calculate condition factors.

Whatever the purpose of the measurement, shrinkage of larvae during collection or fixation can result in many errors. Most ichthyoplankton is preserved in formalin, which results in various degrees of body shrinkage. Studies on the effects of formalin solutions (Blaxter 1971; Hay 1981, 1982; Lockwood and Daly 1975; Parker 1963; Rosenthal et al. 1978; Theilacker 1980; Tucker and Chester 1984) demonstrated that reduction in larval length caused by preservation can be as high as 22% (Baxter 1971; Schnack and Rosenthal 1978), and depends on the initial lengths of the specimens and duration of storage. It should be emphasized that generalized factors have not been easily extrapolated for use in correcting preserved lengths to live lengths for different species of fish larvae (Hay 1982; Schnack and Rosenthal 1978; Theilacker 1980).

I found no shrinkage in larvae placed directly into the ethanol. Reductions in larval lengths for specimens preserved in alcohol reported in previous studies (Radtke and Waiwood 1980; Theilacker 1980; Fowler and Smith 1983), were probably due to the handling associated with the preservation of specimens in these studies. The present results indicate that larval shrinkage occurred upon death. Maximum percent shrinkage was observed in the smallest larvae within 15 min of death. Fifteen minutes is a standard towing time for ichthyoplankton, and many larvae captured with plankton nets are killed in the net, and may have been dead for nearly 15 min by the time they are preserved. Thus, most shrinkage takes place while the fish is still within the net. This source of error is rarely considered in ichthyoplankton field studies, and it means that published larval lengths could be off by as much as 40%.

Accounting for larval shrinkage is important, and the use of otoliths may allow for correction for the effects of death and preservation. Whatever the degree of shrinkage, otolith size can be a reliable estimate of length (Templeman and Squires 1956); especially in larvae (Radtke and Dean 1982). Otoliths may deteriorate if preserved in formalin or in low concentrations or ethanol, but rarely do so if kept in 95% ethanol or pH-adjusted 95% ethanol. Consequently, if properly preserved, otoliths are unlikely to undergo postmortem shrinkage.

In a recent study of shrinkage in larvae of the bay anchovy, *Anchoa mitchilli*, Leak (1986) used the larval length–otolith diameter relationship determined in laboratory-reared larvae to study shrinkage in wild-caught larvae. The present study agrees with Leak's findings, but shows that there are different growth stanzas for cod otoliths which must be taken into account. The standard error (Table 2) is critical for the prediction of fish size from otolith size and it must be less than the percent shrinkage if the fish size–otolith size relationship is to be useful for shrinkage correction. The standard error for small larvae is $< 10\%$ and it would be appropriate to use otolith diameters to correct for the $> 30\%$ shrinkage. The greater variability in otolith size and the smaller degree of shrinkage among older fish may diminish the ability and need to correct for shrinkage. Otolith size–body size inflections occur for intermediate sized larvae, and this would limit the ability to correct for shrinkage. Still, by determining the fish size–otolith size relationship, it becomes possible to predict the size of net collected larvae from otolith diameter.

In conclusion, the structural composition of cod otoliths provides information about the calcification processes and life histories of individual fish. Otolith increment deposition remained daily, in spite of differences in developmental rates due to temperature. Daily, albeit faint, increments were observed in starved cod. Transition zones in the otolith occurred at hatching, yolk-sac absorption and settlement. Thus, microstructural analyses of otoliths allowed accurate ageing and the timing of life-history events. Otolith size was related to fish length, and daily growth patterns may be estimated from the width of individual microincrements. The otolith size–fish size relationship may also be useful in the correction of larval shrinkage. Interpretation of the life-history data recorded in otoliths requires characterization of otolith properties through laboratory experiments. Once the interpretation is clear, otoliths would be invaluable to population, ecological and evolutionary studies, and would supply a new level of knowledge to fisheries biologists.

TABLE 2. Parameters of the relationship between otolith diameter (x) and total body length (y).

Body size	a	(SE)	b	(SE)
< 7 mm	-5.5	0.0015	$6.9(\log x)$	0.0011
7–27 mm	2.3	0.0059	0.86	0.0051
>27 mm	19.1	0.0089	0.02	0.0082

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Humpback Whales (*Megaptera novaeangliae*) Fatally Poisoned by Dinoflagellate Toxin

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During a 5–wk period beginning in late November, 1987, 14 humpback whales, *Megaptera novaeangliae*, died in Cape Cod Bay after eating Atlantic mackerel, *Scomber scombrus*, containing saxitoxin (STX), a dinoflagellate neurotoxin responsible for paralytic shellfish poisoning in humans. We propose a line of evidence to explain how whales, by virtue of their diving adaptations, may be particularly vulnerable to this systemic neurotoxin. Absence of STX in New England waters and shellfish during the episode suggests that the mackerel, representing the northern stock which spawns in the Gulf of St. Lawrence, accumulated the toxin there and delivered it to the Gulf of Maine and Cape Cod Bay in the fall of 1987. These findings challenge common perceptions of the manner in which planktonic toxins move through the food chain, and offer new insights into natural mortality and strandings of marine mammals. It seems appropriate to search for STX and other phytotoxins when investigating marine mammal mortalities.

À partir de la fin novembre 1987 et sur une période de 5 sem, 14 rorquals à bosse, *Megaptera novaeangliae*, sont morts dans la baie de Cape Cod après avoir mangé du maquereau, *Scomber scombrus*, cyontenant de la saxitoxine (STX), une neurotoxine produite par les dinoflagellés et responsable de l'intoxication paralysante des humains par les mollusques. Cet article tente d'expliquer en quoi les baleines pourraient s'avérer particulièrement vulnérables à cette neurotoxine systémique à cause de leurs adaptations liées à leur comportement de plongée. Le fait que les eaux et les mollusques de la Nouvelle-Angleterre ne contenaient pas de STX durant cette période laisse penser que les maquereaux ingérés par les rorquals, qui constituent le stock nordique et fraient dans le golfe du Saint-Laurent, auraient transporté cette toxine dans le golfe du Maine et dans la baie de Cape Cod à l'automne 1987. Ces résultats bouleversent les idées reçues sur la manière dont les toxines planctoniques cheminent dans la chaîne alimentaire et ouvrent de nouvelles perspectives sur la mortalité naturelle et l'échouage des mammifères marins. Il semble indiqué de tenter de détecter la STX et d'autres phytotoxines au cours des recherches portant sur la mortalité des mammifères marins.

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Between November 28, 1987 and January 3, 1988, 14 humpback whales (7 females; 7 males, including the only calf), *Megaptera novaeangliae*, were stranded dead along the beaches of Cape Cod Bay and northern Nantucket Sound, MA. Although not unusual for toothed whales, group mortality of this kind is unprecedented for baleen whales, which come ashore rarely and singly. Based on the nature and timing of the episode, behavioral observations of a terminally-ill animal,

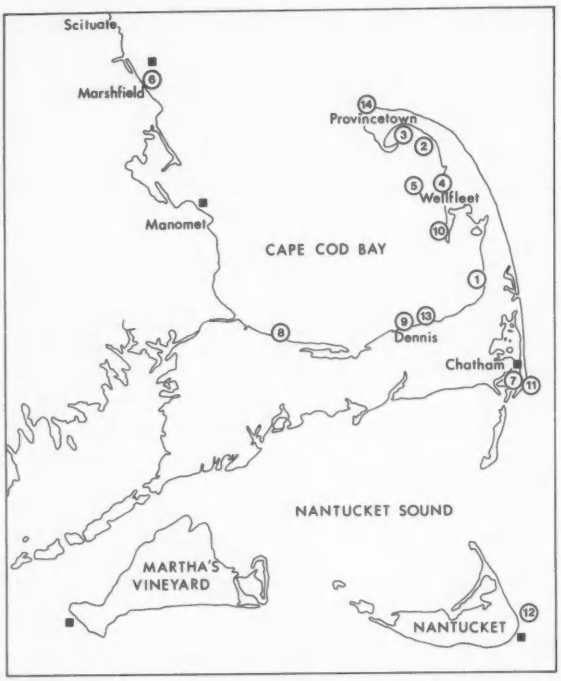

FIG. 1. Location and stranding sequence (numbered circles) of humpback whale carcasses washed ashore in Cape Cod Bay. The event peaked Dec. 15–22, with the landing of whales 4–12. Black squares designate the location of two fin, *Balaenoptera physalus*, and three minke, *B. acutorostrata*, whales cast ashore during the same period.

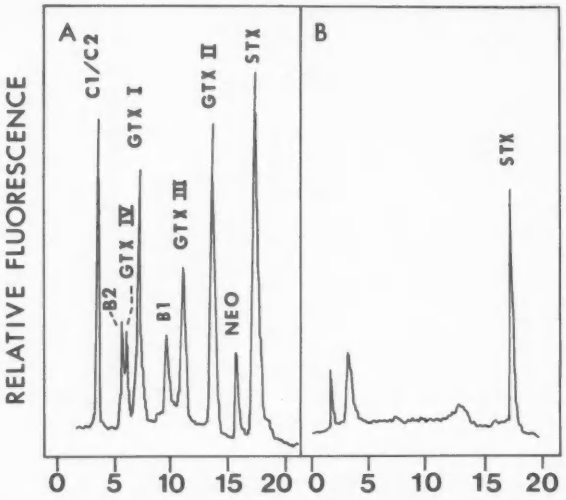

FIG. 2. HPLC chromatograms of paralytic shellfish poisoning toxins. A. Elution profile of a standard toxin mixture containing saxitoxin (STX), neosaxitoxin (NEO), and nine derivatives: B1, B2, C1, C2, GTX (gonyautoxin) I, II, III, IV. B. Elution profile of an extract of mackerel viscera. The toxin in the fish existed only in the parent form.

condition of the carcasses, and laboratory analyses of food-fish and whale tissues, we conclude that the whales died by consuming Atlantic mackerel (*Scomber scombrus*) containing saxitoxin (STX). This neurotoxin, which is the cause of paralytic shellfish poisoning (PSP) in humans, is produced by marine dinoflagellates and certain bacteria (Shimizu 1987). This is the first report of STX accumulation and transfer through a living, commercially important pelagic fish, and of its role in killing marine mammals.

The whales died at sea. Except for one which was towed ashore dead, the carcasses sank to the bottom, were buoyed to the surface by decomposition gasses, and deposited on land by wind, tides, and currents. Hence there was no temporal or geographic pattern to the distributiion of beached whales within the Bay (Fig. 1), and none was fresh when examined; six were moderately and eight extremely decomposed.

Death was quick; one whale observed close to shore seemed to behave normally and 90 min later was dead (C. Carlson, International Wildlife Coalition, Falmouth, MA, pers. comm.). Gross examination of 12 whales, and limited histopathologic analyses on 3, revealed no significant lesions. The carcasses were robust, with abundant hypodermal fat (blubber). Six of 9 stomachs examined contained incompletely digested fish including mackerel, indicating that the animals had been feeding just before they died.

The pattern of deaths prompted the search for an acutely toxic substance. We focused on STX because of its periodic presence in New England shellfish (Shumway et al. 1988). Mackerel caught in Provincetown harbor on Dec. 15, 1987, as well as stomach contents and tissues from the whales were analyzed for STX and its derivatives using standard AOAC mouse bioassay (Association of Official Analytical Chemists 1984). Extracts found to be positive were verified using high performance liquid chromatography (HPLC) (Sullivan and Wekell 1988) (Fig. 2), and by correlating bioassay dose–response curves for serially diluted preparations. Saxitoxin acts by blocking the entry of sodium ions to nerve and muscle cells; selected samples were tested for this effect using neuroblastoma tissue culture (Kogure et al. 1988). During the outbreak, plankton samples were collected in the vicinity of feeding whales near Provincetown, using vertical tows with a 30 μm mesh plankton net. Samples were concentrated by centrifugation, freeze-thawed three times to lyse the cells, and extracted with 0.1 *M* HC1.

Saxitoxin was universally present in viscera, especially liver, of mackerel caught at the time and place the whales were feeding, and in others collected between the Isles of Shoales, NH, and Pt. Judith, RI, (approximately 80 km north and south of the study area) on Dec. 16 and 18, 1987. Only STX was detected, and not any of its 11 derivatives (Fig. 2). Five analyses of composite tissues from 17 fish showed STX in liver at a mean concentration of 153 μg/100 g. Average concentration in 4 fish was 52.3 μg STX/100 g of viscera (range 40.2–71.2 μg/100 g), equivalent to a total body burden of 80 μg STX/kg fish. No toxin was detected in fish muscle, a reassuring finding in a commercially valuable fish. Pacific mackerel, *Scomber japonicus*, tested as controls, contained no STX.

Extracts from 3 of 8 whale kidneys, 4 of 7 livers, and the contents from 7 of 9 stomachs (macerated flesh, bones, and fluid) caused mortality characteristic of STX in mice; control liver samples ($n = 21$) from two humpback whales, one minke whale, *Balaenoptera acutorostrata*, one fin whale, *B. physalus*, four common dolphins, *Delphinus delphis*, and 13 bottlenose dolphins, *Tursiops truncatus*, that died unrelated to this incident showed no such toxicity. One each of the positive kidney and liver samples from the whales was also tested and found

positive in the tissue culture assay for sodium channel blockage (Kogure et al. 1988). However, HPLC analysis showed no STX peak. Given the certainty with which STX was present in fish, we assume that once digested or in tissues, STX was transformed into compounds that still elicited characteristic signs in mice, but that were not identifiable by the standard HPLC protocol, as has been observed in shellfish (Sullivan et al. 1983).

How much toxin did the whales consume? We calculate that a whale eating 4% of its weight in mackerel daily (Sergeant 1969) would have consumed STX at a dosage of 3.2 μg/kg of body weight, enough to cause illness perhaps, but not death in humans for whom the minimum lethal oral dose is estimated to be 7–16 μg/kg (Schantz et al. 1975). There are no data that can be used to determine the effects of this amount of toxin on whales. However, conventional pharmacological dogma suggests that larger animals require proportionally less for the same effect (Casarett 1975). Furthermore, lethal dose estimates are based on a single administration of toxin, and not on cumulative effects of sublethal concentrations, as would occur in actively feeding whales.

Beyond this are physiological factors that are expected to increase the effect of a given dosage of STX in the whales. Thirty percent of the mass of a humpback whale is poorly perfused, metabolically inactive blubber (Ash 1957). Water-soluble STX would by-pass this depot and concentrate in more physiologically sensitive tissues. During a dive, marine mammal blood is channeled to the heart and brain (Ridgway 1972), thus funneling the toxin to those organs while limiting access to liver and kidney where metabolism and elimination occur. The same processes that lead to reversible peripheral nerve impairment, mild hypothermia and muscle fatigue in poisoned humans (McFarren et al. 1960) may cause a whale to lose control over vital peripheral heat-conserving mechanisms (Ridgway et al. 1974) or affect its ability to return to the surface to breathe. The unusual sensitivity of a cetacean's respiratory system to anesthetics (Ridgway and McCormick 1971) and the importance of ionic metabolism in their cortical neurons (Glezer et al. 1987) further suggest that the respiratory center would be a vulnerable target for a centrally active neurotoxin such as STX (Borison et al. 1980).

Apart from its implications for whales, humans, and other consumers of fish, the discovery of STX in mackerel challenges our understanding of accumulation and retention of the toxin in fish. No STX was detected in New England shellfish, or in plankton sampled in the vicinity at the time of the outbreak, nor did the plankton assemblage contain algal species associated with PSP. The toxin in mackerel could have been a metabolite of endogenous bacteria (Kodama et al. 1988). More likely the fish, representing the northern stock which spawns in the Gulf of St. Lawrence (Anderson and Paciorkowski 1980), accumulated STX from planktonic sources there (White 1982; J. Martin, Canada Department of Fisheries and Oceans, Biological Station, St. Andrews, N.B., pers. comm.) converted the STX derivatives to the parent form (Shimizu and Yoshioka 1981), retained it as some shellfish do (Schantz and Magnusson 1964), and were already contaminated when they entered Cape Cod Bay during October–November. Burdened by STX at levels toxic to other fish (White 1981), the mackerel were perhaps easier prey.

Given the history of PSP in the region (Prakash et al. 1971), it is likely that related poisonings are broader in scope than we present. In fact, during the same period, 5 decomposed carcasses of 2 other whale species washed ashore on Cape Cod (Fig. 1). And in November 1988, a year after the original discovery, we found STX in mackerel from the stomach of a dead humpback there. Toxic dinoflagellates are distributed worldwide, and associated poisonings may have occurred (White 1984) unnoticed against the background of occasional strandings. It now seems appropriate to search for STX and other phytotoxins when investigating these events.

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Minimum Size Regulations and the Implications for Yield and Value in the Canadian Atlantic Halibut (*Hippoglossus hippoglossus*) Fishery

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Neilson, J. D., and W. R. Bowering. 1989. Minimum size regulations and the implications for yield and value in the Canadian Atlantic halibut (*Hippoglossus hippoglossus*) fishery. Can. J. Fish. Aquat. Sci. 46: 1899–1903.

The effect of a minimum size regulation on yield and value per recruit in the Canadian Atlantic halibut fishery was examined. The model indicated that under most scenarios, the size limit would not result in increased yield per recruit. In general, yield per recruit was more sensitive to fishing mortality than age of first entry to the fishery. While reduced yields were usually associated with the minimum size limit, the value per recruit increased with increasing age at entry to the fishery until age 7. The changes in value per recruit reflected the size composition of landings following the imposition of the size limit and the different values associated with various size categories. Both yield and value per recruit were sensitive to the choice of the natural mortality rate.

On a observé l'effet de l'imposition d'une limite de taille sur le rendement et la valeur par recrue dans le cas de la pêche canadienne du flétan atlantique. Le modèle a révélé que, dans la plupart des cas, l'imposition d'une limite de taille n'occasionnerait pas une augmentation de rendement par recrue. En général, le rendement par recrue est plus sensible à la variable mortalité par capture qu'à la variable âge d'entrée dans la phase exploitable. Bien que des rendements diminués étaient généralement associés à la limite de taille minimale, la valeur par recrue a augmenté proportionnellement à l'âge d'entrée dans la phase exploitable, jusqu'à 7 ans. Les variations de la valeur par recrue reflétaient la composition par taille des prises débarquées suivant l'imposition d'une limite de taille et les valeurs différentes associées aux diverses catégories de taille. Le rendement et la valeur par recrue étaient tous deux touchés par le choix du facteur taux de mortalité naturelle.

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Atlantic halibut (*Hippoglossus hippoglossus*) is the most highly-valued groundfish species (per unit weight) on the Canadian Atlantic coast. Landings of Atlantic halibut in 1986 were 3 555 t, valued at $14,870,000. While longlines are the most commonly-employed gear type in the Scotian Shelf and Gulf of St. Lawrence areas, trawl catches off Newfoundland constitute almost as large a fraction of total landings as do those of longline gear (Neilson et al. 1989). With enhanced interest in the longline fishery of Atlantic halibut following the introduction of the comparatively more efficient circle hook in the early 1980's (Neilson et al. 1987), there has been an increasing need for management measures to conserve the stocks.

As part of the assessment of the utility of a proposed 81-cm (32") minimum size limit, a study of the survival of trawl and long-line caught fish was completed in 1987 (Neilson et al. 1989). Those authors examined the survival of trawl- and long-line-caught Atlantic halibut using a live holding facility onboard a research vessel. Attempts were made to simulate commercial practices during the fishing operations, although commercial set durations and handling time of the catch were often longer. Only 35% (70 of 198 total) of fish less than 81 cm survived more than 48 h after capture in bottom trawl sets of 120-min duration, whereas 77% of longline-caught fish less than 81 cm survived (10 of 13 total). In light of those results, the minimum size limit was adopted in 1988 on an interim basis only, subject to an analysis of the effectiveness of the minimum size limit in terms of fishery yield. An identical size limit has been in place since 1973 for the fishery on the closely-related Pacific halibut (*Hippoglossus stenolepis*) (Myhre 1974). The assessment of the effectiveness of the minimum size regulation for Atlantic halibut is the subject of this contribution.

Recognizing that prices differ with market size of halibut (with intermediate-sized fish commanding the highest values) and that imposition of a minimum size regulation could affect the size composition of landings, an examination of the utility per recruit was undertaken in addition to the more traditional yield per recruit (biomass). The technique employed used value as the utility function (Die et al. 1988). The analyses presented are for the stock occurring in the North Atlantic Fisheries Organization Statistical Divisions 4VWX, 3NO and Subdivision 3Ps (including the Grand Bank and St. Pierre Bank off Newfoundland and the Scotian Shelf, Fig. 1), the largest of the two Atlan-

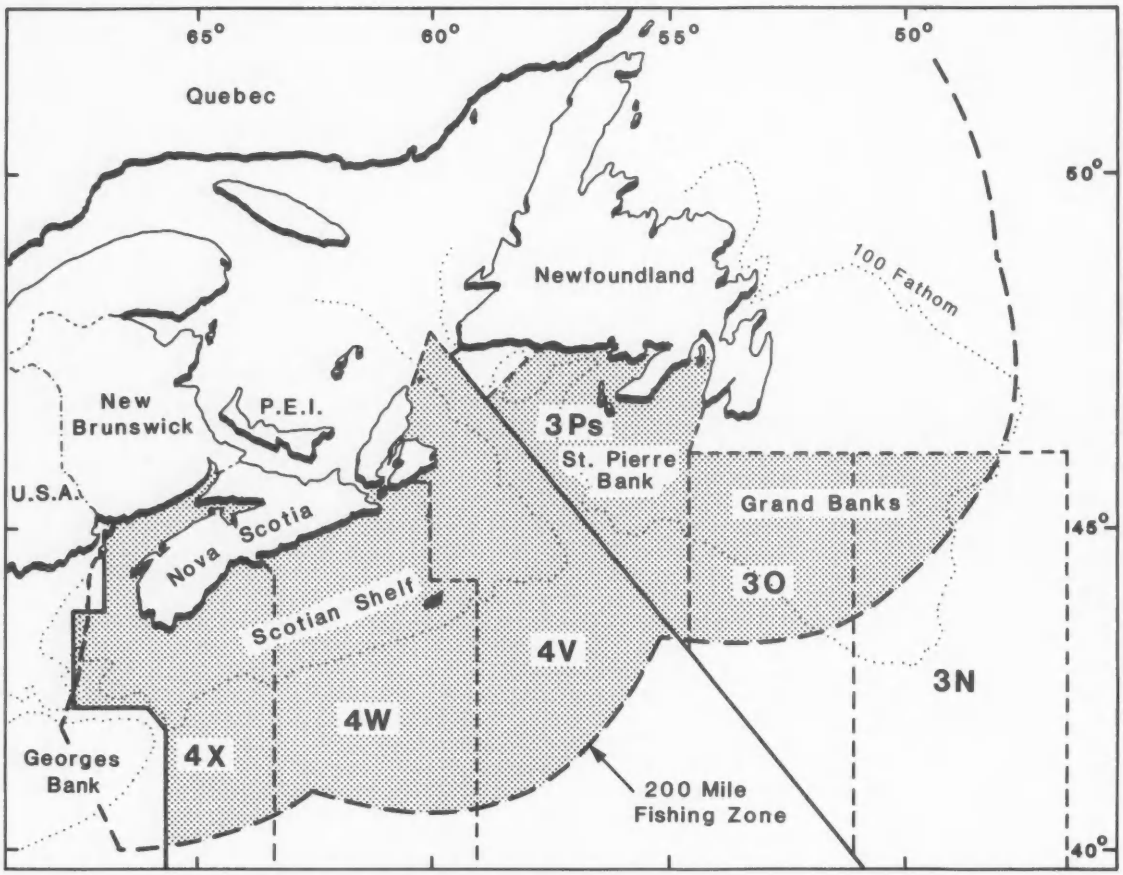

FIG. 1. The coast of Atlantic Canada, showing the North Atlantic Fisheries Organization Statistical Boundaries and the bathymetric features referred to in the text. The study area is stippled and encompasses the range of the stock thought to occur on the Scotian Shelf and southern Grand Banks.

tic halibut stocks thought to occur in Canadian waters (Neilson et al. 1987). While studies of the impact of minimum size regulations have been completed for other species, our work is the first examination of the effectiveness of a management measure for the Atlantic halibut fishery, which was previously unregulated. Also, past attempts to incorporate the effects of release mortality on yield per recruit have often employed a range of mortality rates associated with capture and release, as such data were unknown (see, for example, Waters and Huntsman 1986). In our case, we have empirical data from the experiment described in the preceding paragraph (Neilson et al. 1989) which were incorporated into the analyses.

Methods

The model of Ricker (1945) was used for estimating the optimum age of entry into the fishery, as growth and mortality are treated as age-specific. This technique was also used to support the choice of the 81-cm size limit currently in effect for Pacific halibut (Myhre 1974). The yield, Y, obtained each year from the hypothetical population is:

$$Y_t = 1/2\, N_t \bar{W}_t [1 + \exp^{(-F_t S_t (1 - K + KR) - M_t + g_t)}] F_t S_t R$$

where:

N_t is numbers at age;
$\bar{W}_t$ is mean weight at age;
F_t is fishing mortality;
M_t is natural mortality;
S_t is the gear selection ratio during t and takes values $0 \leq S_t \leq 1$;
K is the survival rate of released fish (undersized fish returned to the water, and takes values $0 \leq K_t \leq 1$;
R is the retention factor which is 0 if t is less than the age of entry (age corresponding with the minimum size regulation), and
g_t is the instantaneous growth rate during the period t.

As noted by Myhre, the exponential term controls change in population biomass during t, and can be viewed as the "production" term. Removals from the fishery are governed by F_t, S_t, K, and R. No yield to the fishery will accrue when $R = 0$ (minimum size is in effect and undersized fish are caught) but the numbers of fish that remain will decline according to M and K. The total yield expected from a year-class is the sum of the annual yields during its period of vulnerability to the fishery.

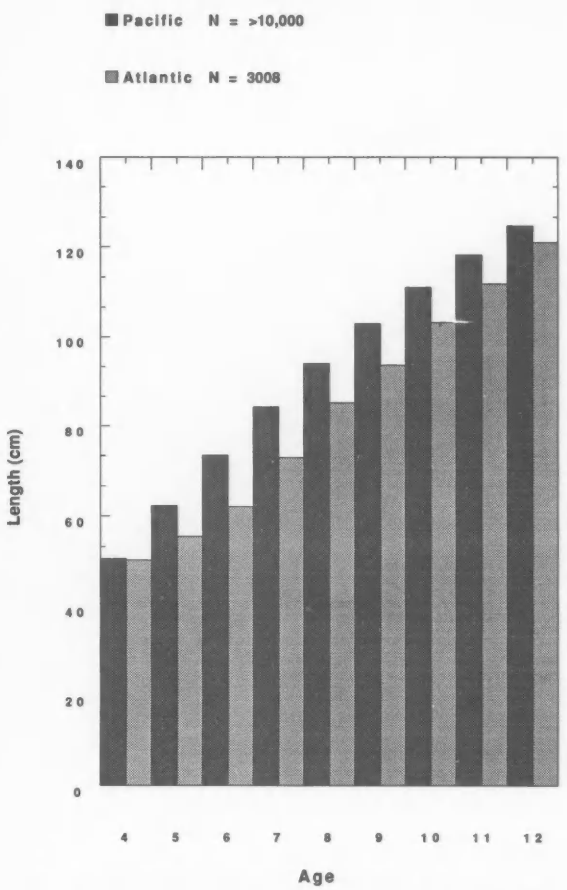

FIG. 2. Comparison of length (fork length) at age of Atlantic and Pacific halibut. Atlantic data were from Department of Fisheries and Oceans collections made in the study area from 1986 to 1987. The Pacific data are from Myhre (1974).

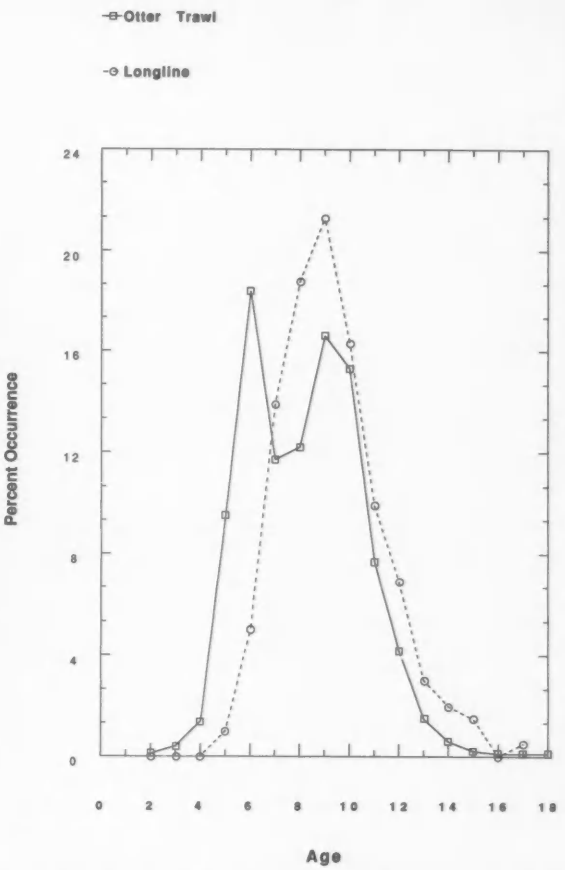

FIG. 3. Age composition of otter trawl and longline-caught Atlantic halibut. See text for explanation of the derivation of the curves.

A constant initial biomass is assumed. However, recruitment is expected to vary with environmental and fishery-related factors. A fixed selection curve is employed but more realistically, with the imposition of a size limit, fishermen might be expected to shift their fishing grounds to avoid concentrations of small fish. Retention is also assumed to be "knife-edged", with all fish at the age of first entry to the fishery kept.

Length-frequency and age information ($N = 3008$) obtained from sampling the commercial fishery throughout the management unit was used to derive estimates of catch-at-age for both gear types and mean length-at-age. A length-weight relationship (Bowering 1986) was used to calculate mean weight-at-age. Such values were judged to be accurately estimated for ages 4–12 inclusive. However, for ages 3 and 13 and older, it was necessary to fit a von Bertalanffy growth curve to the available data, and extrapolate. The von Bertalanffy equation was $l_t = 201.5(1\text{-}e^{-0.0852026(t-0.640041)})$.

In the absence of data pertaining to size selection at age for the Atlantic longline fishery, it was assumed that data from the Pacific coast longline fishery were applicable. Such an assumption seemed reasonable as a first approximation given the congeneric status of both forms, and the similarity of the gear employed. However, Pacific halibut grow somewhat faster than Atlantic halibut (Fig. 2), hence the Pacific halibut selection factors were lagged by 1 yr. Based on an exchange of otoliths between age readers responsible for the Pacific and Atlantic age determinations, such differences at age did not appear to be due to inconsistencies in techniques. The selection ratios for the Atlantic coast otter trawl fishery were based on an evaluation of the proportion of fish at age taken in the longline and otter trawl fisheries (Fig. 3). Typically, the proportion of younger fish taken by otter trawl gear was greater than that taken by longline gear. In view of the uncertainty regarding gear size selection at age, two scenarios were developed. In both cases, selection by the otter trawl gear was taken as the ratio of proportion of numbers caught at age in otter trawl:longline multiplied by the longline selection ratio, and gave values of 0.113 at age 3, 0.227 at age 4, 0.331 at age 5, 0.464 at age 6, 0.654 at age 7, and 0.876 at age 8. However, the first case assumed that selection was 1.0 at age 9 and thereafter (flat-topped). In the second, the values were calculated by the ratio method throughout the range of exploited ages, as done for ages 3 through 8 described above. This method yielded a roughly dome-shaped curve, a result also found in Pacific halibut studies (Myhre 1969). We concluded that the period of vulnerability to the fishery was from ages 3–12 inclusive (Fig. 3). However,

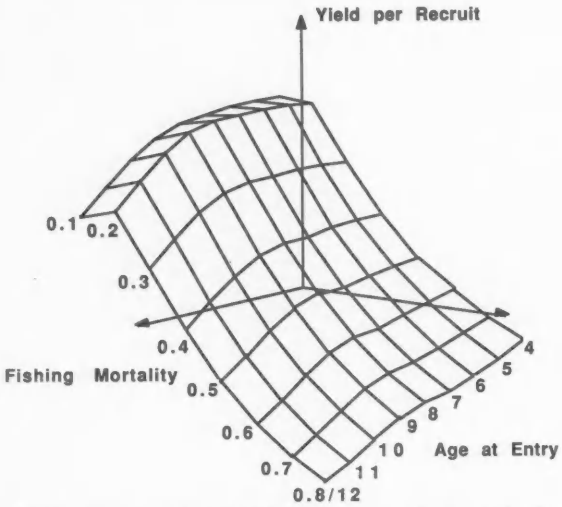

FIG. 4. Response surface for Atlantic halibut yield per recruit with varying age of entry and fishing mortality. The selection curve was assumed to be flat-topped, and natural mortality was 0.1.

to examine the sensitivity of our conclusions with regard to period of vulnerability, we also included a further variation wherein fish were assumed to be exploited from ages 3–20 inclusive.

To reflect the mixed gear nature of the fishery, composite values of K were determined by age for longlines and otter trawls, weighted by the proportion of catch by the gear types from 1985 to 1987. Since sampling of fish older than age 15 was inadequate, K was assumed constant thereafter. The resultant K values are shown below:

Age	K
3	0.354
4	0.354
5	0.447
6	0.531
7	0.672
8	0.691
9	0.678
10	0.664
11	0.679
12	0.695
13	0.706
14	0.732
15–20	0.754

The analyses were completed using M's of 0.1 and 0.175 and F ranging from 0.1 to 0.8. The latter value of M was also chosen by Myhre (1974).

Data on the value of halibut were obtained by multiplying the mean weight at age by the conversion to gutted head-on weight (0.85), the normal landed condition. These values were grouped by market category (1–7 lb = "snapper", 7–12 lb = "chicken", 12–60 lb = "medium", 60–125 lb = "large", > 125 lb = "whale") and multiplied by the mean monthly value for 1987, weighted by the relative contribution of each monthly landing to the total landings by market category. In this fashion, the effect of including extreme values often associated with short-term variations in supply was reduced. The resulting values per pound were as follows: snapper, \$2.04; chicken, \$2.19; medium, \$3.59; large, \$3.02; whale, \$2.50.

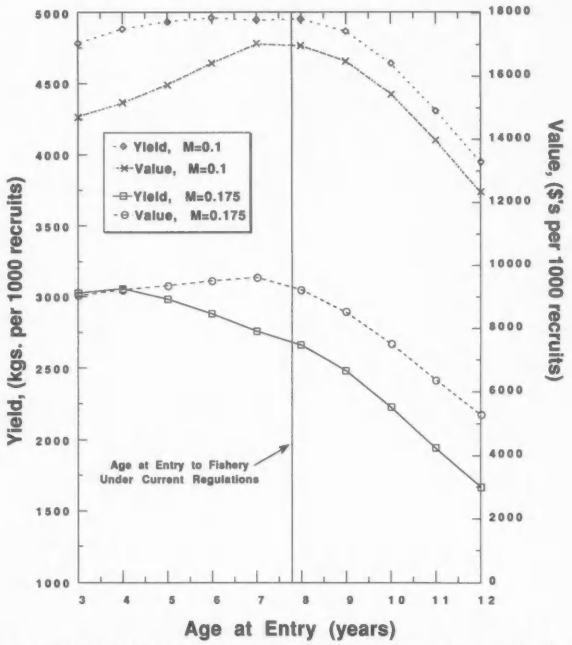

FIG. 5. Yield and value per recruit versus age of entry to the Atlantic halibut fishery. Natural mortality (M) is 0.1 or 0.175, fishing mortality was assumed to be 0.3, and the selection curve was assumed to be flat-topped.

Results and Discussion

An example of results obtained in the yield per recruit analyses is shown on Fig. 4, which shows the yield for the model when $M = 0.1$. In that instance, yield is maximized at an F of 0.2, and an age of entry to the fishery of 6 yr. With increasing fishing mortality and age of entry, yields decrease markedly. In general, other analyses indicated that results were sensitive to the choice of M, the range of ages exploited and the shape of the selection curve. Therefore, a range of the above variables was considered and the percent increase/decrease in yield and value per recruit compared with the situation where no minimum size limit exists (summarized below). For illustrative purposes, the fishing mortality is taken to be 0.3. The age of entry was 8 yr, corresponding to the length of the minimum size now in effect.

	Increase (+)/decrease(−) Yield (%)	Value (%)
M = 0.1, ages 3–20, flat-top selection	+5.9	+15.4
M = 0.1, ages 3–20, domed selection	+5.0	+13.5
M = 0.1, ages 3–12, flat-top selection	−5.8	+6.3
M = 0.1, ages 3–12, domed selection	−4.5	+5.9
M = 0.175, ages 3–20, flat-top selection	−9.0	+2.2
M = 0.175, ages 3–20, domed selection	−7.9	+2.0
M = 0.175, ages 3–12, flat-top selection	−17.8	−4.9
M = 0.175, ages 3–12, domed selection	−15.7	−4.2

Figure 4 also shows the interrelationship of fishing mortality and age at entry on fishery yield. The effect of varying age at entry seems to be of secondary importance relative to fishing effort, as indicated by the rapidly decreasing slope along the fishing mortality axis after $F = 0.2$, compared with the relatively flat surfaces along the axis of age at entry.

Under all but the most extreme of scenarios illustrated in the text table above, the minimum size limit reduces yield per recruit. The minimum size regulation resulted in increased yield only when M was comparatively low and a comparatively wide range of ages were available for exploitation. In comparison, the International Pacific Halibut Commission used $M = 0.175$ in a similar analysis (Myhre 1974). Hence, it seems unlikely that the imposition of a minimum size regulation will result in increased yield per recruit in the Atlantic halibut fishery. Similar results were obtained for other plausible values of F. This result is due to the comparatively long period that halibut are vulnerable to exploitation: a released fish may be removed by natural mortality before it is again harvested.

However, the changing value of the landings resulting from differences in market size composition gives a different impression of the utility of the size regulation (see text table and Fig. 5). The figure shows both value and yield at a fishing mortality of 0.3, and illustrates the effect of varying natural mortality. When $M = 0.1$, yield is roughly stable through ages of entry of 4 through 8, but value is increasing up until age 7. However, when $M = 0.175$, yield declines from age 4, yet values again increase until age 7, although the incremental gains are smaller than was the case when $M = 0.1$. The value analysis illustrates the potential danger in relying solely on the traditional measure of biomass as an indicator of the utility of the management measure. The figure also demonstrates the sensitivity of the model to the choice of the rate of natural mortality. Unfortunately, as is the case for most other exploited fish populations, M is poorly defined.

A limitation of the analyses presented here is that only a deterministic view of the model is provided. Recent advances in the application of yield per recruit modeling have explicitly included parameter uncertainty (Restrepo and Fox 1988). An example of the uncertainty present in our analysis is that Neilson et al. (1989) found that K was 0.35 and 0.77 for bottom-trawl caught and longline-caught halibut, respectively. The corresponding approximate 95% confidence intervals based on the binomial distribution are 0.287–0.420 and 0.540–1.0, respectively. A 10% change in yield per recruit was associated with the upper and lower limits of the approximate 95% confidence interval (calculated when $F = 0.3$ and $M = 0.1$). Furthermore, a more realistic approach might include scope for variation in K with length, as Neilson et al. (1989) have indicated that those factors do indeed covary. Another improvement to the model would include the inclusion of cost information, as suggested by Die et al. (1988). However, given the number of assumptions already implicit in the analyses, the data were not deemed adequate to warrant such refinements to the model at present.

Myhre (1974), in his related analysis of the Pacific halibut fishery, indicated that higher yields would result from the introduction of an 81-cm size limit. However, in that instance, K was assumed to be 0.32. The low value of K relative to Atlantic halibut fishery reflects the comparative dominance of the hook and line fishery on the Pacific coast. Other differences between Myhre's analysis and ours are likely attributable to differences in weights-at-age and selection ratios. The latter difference follows from the greater proportion of landings taken by otter trawlers in the Atlantic fishery.

On the basis of the value and yield analyses presented here, it is probable that the 81-cm size limit for Atlantic halibut will result in slight losses of yield, ameliorated by increased value of the landings. The extent of benefits expected from the introduction of the minimum size regulation depends on the rate of natural mortality. However, changes in yield or value per recruit will not occur immediately. Both will decrease in the short run, reflecting the cessation of removals of small fish while catches of larger fish are initially unchanged, before they increase again in the long run. This is particularly true for a long-lived species such as halibut.

As noted by Ricker (1945), optimization of a fishery based on yield per recruit considerations alone is only part of a rational scheme for management. Even when value data are included, there are other factors worth thought. One important consideration is what effect a change in number of spawners would have on the abundance of recruits, and how such changes would affect the rate of growth of the stock. In the case of the Atlantic halibut fishery, such data are not available at present.

Acknowledgments

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Interactive Effects of Geophysically-Forced Sea Temperatures and Prey Abundance on Mesoscale Coastal Distributions of a Marine Predator, Atlantic Cod (*Gadus morhua*)[1]

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Rose, G. A., and W. C. Leggett, 1989. Interactive effects of geophysically-forced sea temperatures and prey abundance on mesoscale coastal distributions of a marine predator, Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 46: 1904–1913.

Geophysically-forced sea temperatures and prey abundance interactively regulated mesoscale (10's to 100's of km) inshore Atlantic Cod (*Gadus morhua*) distribution patterns in the northeastern Gulf of St. Lawrence, in both 1985 and 1986. Cod were located most often at temperatures within the range 0–5°C. However, when mean densities of their chief prey (capelin, *Mallotus villosus*) were high (>100/10^{5}m^3) within the coastal zone, cod frequented waters having temperatures similar to those at which this prey were aggregated, within the broader range of temperatures −0.5 to 8.5°C. The proportions of cod occupying sea temperature strata were significantly and positively correlated with those of capelin (r = 0.64, slope = 0.6, df = 86), in both 1985 and 1986, under two conditions: (1) sea temperatures within the range 1–9°C; and (2) mean capelin densities > 100/10^{5}m^3. Under conditions of lower mean capelin densities, cod distributions were independent of those of capelin. At temperatures outside the range 1–9°C, cod and capelin densities were not correlated, regardless of capelin densities.

Les températures de l'eau liées à des phénomènes géophysiques et l'abondance des proies régissaient de façon interactive les schémas de répartition à échelle moyenne (dizaines à centaines de km) de la morue franche (*Gadus morhua*) dans le nord-est du golfe du Saint-Laurent, en 1985 et 1986. Les morues se retrouvaient le plus souvent à des températures de l'ordre de 0 à 5°C. Cependant, lorsque la densité moyenne de leur proie principale, le capelan (*Mallotus villosus*) était élevée (>100/10^5 m^3) dans la zone côtière, les morues fréquentaient des eaux présentant des températures similaires à celles auxquelles ces proies se rassemblaient, dans une plage plus large de température de −0,5 à 8,5°C. Les proportions des morues occupant les strates de température de l'eau de mer offraient une corrélation positive significative avec celles du capelan (r=0,64, pente = 0,6, FR = 86), en 1985 et 1986, lorsque deux conditions étaient réunies : 1) une température de la mer dans la plage 1–9°C; (2) une densité moyenne de capelan de >100/10^5 m^3. Lorsque la densité des capelans était inférieure, la répartition des morues était indépendante de celle des capelans. Hors de la plage de température 1–9°C, les densités de capelans et de morues n'étaient pas corrélées, quelle que soit la densité des capelans.

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The geophysical forcing of the environment limits the geographic range of many species (Partridge 1978; Krebs 1978). In marine coastal environments, geophysical forcing by winds, tides, and currents is highly variable across a wide range of temporal and spatial scales (hours to many years, metres to hundreds of kilometres). Some variability is periodic. For example, tidal forces cause predictable oscillations of temperature, salinity, depth and light. Other variability is less regular. Wind-driven upwellings of colder deep waters at times replace warmer surface waters in nearshore areas. Alongshore currents having cross-shore upwelling components can cause temperature declines of as much as 10°C within 24 h (Csanady 1982; Rose and Leggett 1988a), in addition to changes in salinity (Rose and Leggett in press a) and biotic factors (Frank and Leggett 1982). Relatively immobile animals inhabiting coastal zones are adapted to sustain environmental variability (e.g., Connell 1961; Theede 1973). In contrast, highly mobile migratory fish that exploit coastal zones often move in synchrony with favorable physical conditions (e.g., Lindquist and Hannerz 1974; Rose and Leggett 1988b). These migrations reduce the environmental variability to which the animal is exposed (Leggett 1985).

In geophysically-forced coastal environments, water temperature often limits fish distribution (e.g., Fleming and Laevastu 1956; Laevastu and Hela 1970; Leggett 1977). For most species, there are temperature limits beyond which individuals are rarely encountered (e.g., Coutant 1977; Scott 1982). Moreover, most species frequent a much narrower temperature range than that which delimits their distribution. This narrower range has been termed "preferred" (e.g., Pitt et al. 1956; Reynolds 1977; Scott 1982), or "optimum" (Laevastu and Hayes 1981).

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However, we believe these usages lead to confusion between field observations and experimentally determined thermal preferenda (sensu Fry 1947). Hence, in this paper we employ "modal" to describe the temperature range at which free-ranging fish are most frequently located.

The predictability of fish distributions relative to sea temperature, and to the causal geophysical forcing, has not gone unnoticed by fishermen, who for many years have used empirically derived "rules of thumb" to predict fishing success and, at times, to guide fishing effort (Thompson 1943). More recent "temperature directed fisheries" employ these rules to guide major fishing activities in relation to sea temperature (Laevastu and Hayes 1981). However, the success of temperature directed fisheries has always been variable (see Cendrero 1965). Not all waters having the "correct" temperatures contain fish. Also, although it is indisputable that when assessed over long time periods, fish are more frequently encountered within well defined temperature ranges, at times fish may be located at much more extreme temperatures. These conditions have led some fishermen to doubt the reliability of using temperature as a single-factor predictor. For example, with regard to Atlantic cod (*Gadus morhua*), Cendrero (1965) noted that "a preferred temperature would not mean necessarily the presence of cod if other preferred conditions were lacking."

Fish distribution is unlikely to be determined solely by geophysical conditions (Laevastu and Hayes 1981). Biotic factors, in particular the distribution and abundance of prey, may also determine their distribution in space and time. Prey may be abundant in waters having temperatures outside a predator's modal range (Templeman 1965; Coutant 1985; Lilly 1987). Under these conditions, there is evidence that free-ranging predators locate themselves at more extreme temperatures, in proximity to the abundant prey (Lee 1952; Fleming and Laevastu 1956; Woodhead and Woodhead 1965). Neill and Magnuson (1974) reported similar results from laboratory experiments. This led us to hypothesize that the limits of distribution of free-ranging fish would be determined by geophysical factors (e.g., sea temperature), but within those limits their distributions would be determined by the interacting influences of modal sea temperatures and prey density.

In this paper, we investigate the effects of sea temperature and prey (capelin, *Mallotus villosus*) densities on the distributions of Atlantic cod. Cod prey heavily on capelin over most of their range in the north Atlantic (Hansen 1949; Powles 1958; Brunel 1965; Minet and Perodou 1978; Lilly and Fleming 1981; Waiwood and Majkowski 1984). In this region, during the spring and summer, cod are rarely located where temperatures exceed the range of -0.5 to 8.5°C (Thompson 1943; Rose and Leggett 1988b). The modal range of temperatures frequented by cod has been reported to be much narrower (e.g., 0–4°C, Anonymous 1988; 0–5°C, Rose and Leggett 1988b).

The capelin prey of cod frequent a much wider range of temperatures, and are at times abundant at temperatures both outside the modal temperature range of cod, and the broader range which delimits cod distributions. For example, during the winter and spring, capelin may aggregate where temperatures are well below -1°C (Lilly 1982; L. Dickie and G. A. Rose, unpublished data). During the onshore spawning migration in early summer, the more pelagic capelin undertake vertical migrations from deeper waters having temperatures within the modal range of cod to surface waters where temperatures at times may exceed 12°C — well outside the range where cod are found (Rose and Leggett 1988b). Despite these apparent differences in the temperature ranges frequented by the two species, capelin are a major year-round prey of cod in some areas (e.g., Minet and Perodou 1978) and are virtually their sole prey during the spring and summer period of the inshore migration (Hansen 1949; Templeman and Fleming 1956; Lear 1984; Lilly 1987; our unpublished data).

We hypothesized that cod densities would "track" those of their highly mobile capelin prey within the thermal environment defining their overall distribution. We chose sea temperature as the environmental index because it is widely considered to be the most important geophysical determinant of fish distribution (possibly as a proxy for accompanying currents and salinities) (Fleming and Laevastu 1956; Leggett 1977; Rose and Leggett 1989a), and because cod and capelin distributions have been shown to reflect underlying dynamic interactions between their respective local densities and sea temperature (Rose and Leggett 1988b, 1989b).

We tested three hypotheses on the effects of sea temperatures and prey densities on cod distributions: (1) that cod would frequent sea temperatures within a consistent modal range when overall prey densities were low; (2) that when prey abundance exceeded some threshold, cod would congregate at temperatures where prey aggregated; and (3) that under the conditions defined in 2 above, cod densities would be positively correlated and proportional to those of their prey.

Methods

This study was conducted at Brador Bay (51°25′N, 57°15′W) in the northern Gulf of St. Lawrence (Fig. 1). Detailed descriptions of the study area and its meteorological and oceanographic regimes and inshore cod fisheries are given in Rose and Leggett (1988a,b, 1989a). Capelin migrate inshore annually at Brador Bay during late June and July, at which time they are preyed upon by migratory cod of the northern Gulf (Lear 1984; Rose and Leggett 1988b). During this period, capelin may be found throughout the study area, although the central portion of Brador Bay, which is not adjacent to any known spawning grounds, seldom contains high capelin densities (Fig. 1). This distribution of capelin and of their spawning areas has persisted for many years (G. Labadie and L. Hobbs, commercial fishermen, Brador Bay, Quebec, personal communications).

We compared predator and prey density distributions at mesoscales (10's–100's of km), which are comparable in magnitude to the dimensions of the study area. These scales were determined by the scale of the relevant temperature strata. At these scales, coastal sea temperature conditions are forced by wind-driven currents and upwelling and downwelling (Rose and Leggett 1988a), a condition typical of coastal Atlantic Canada (Thompson 1943; Templeman 1966; Yao 1986; Taggart and Leggett 1987; Schneider and Methven 1988).

Cod and capelin densities have been shown to be positively correlated at mesoscales (Rose and Leggett 1989b). Hence, the signs of significant correlations between cod and capelin distributions relative to mesoscale geophysical features were expected to be positive. Correlations of densities measured at smaller scales (km) may be negative (Rose and Leggett 1989b).

The distributions and abundances of cod and capelin were assessed acoustically on a daily basis from July 12 to August 2, 1985, and from June 13 to August 4, 1986, at Brador Bay (Fig. 1). We used a Biosonics, Inc. dual beam 120 kHz echosounding system with model 121 echo integrator to assess relative fish densities. Cod and capelin were discriminated by

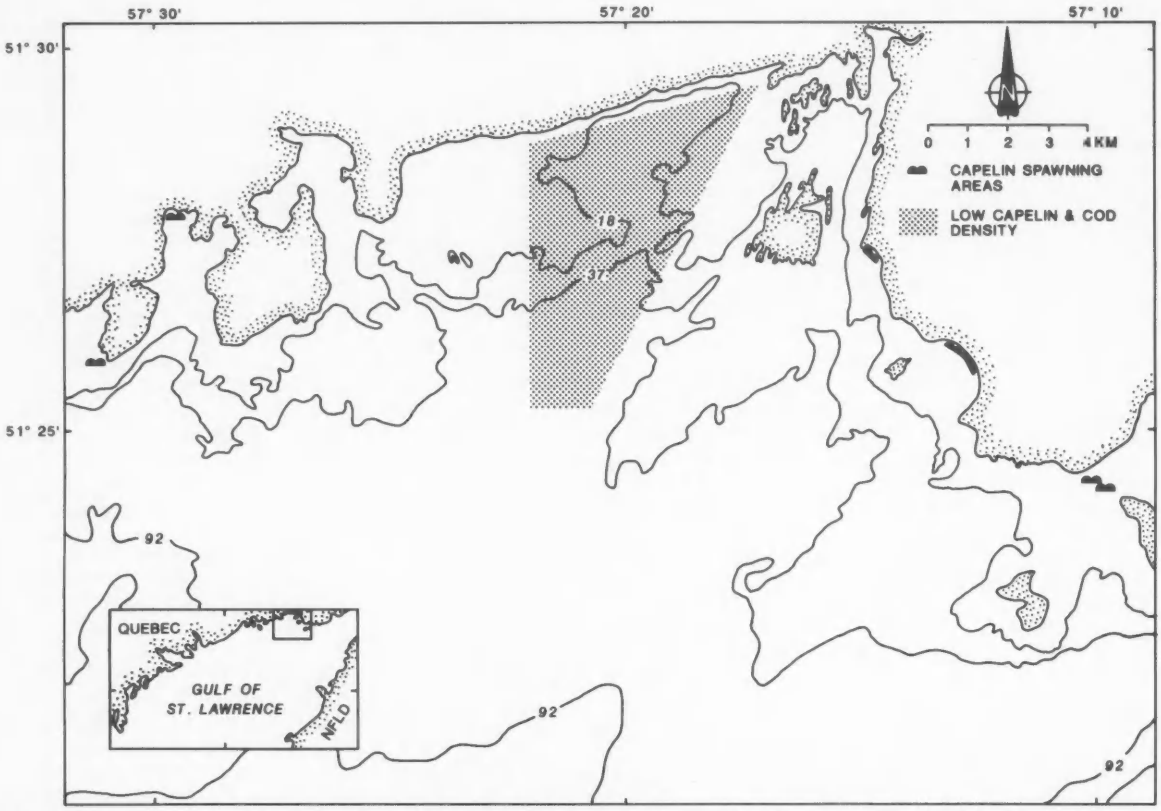

FIG. 1. Brador Bay study area indicating locations of known capelin spawning areas, and the zone where densities of capelin (and cod) are seldom high. Isobaths in m.

employing the quadratic discriminant functions developed for this purpose by Rose and Leggett (1988c). An average of 50 (range 21 to 116) acoustic transects of 1 km length were run daily between 0600 and 1600 h. Cod were not actively pursuing capelin then, and with a few exceptions, their aggregations were discrete (Rose and Leggett 1988b, 1989b). All spatial and depth ranges of the study area were sampled. Transect depths ranged from 10 to 100 m. For each transect, densities were stratified by depth into 10 m blocks. Acoustic estimates of the densities of cod and capelin were determined for each 10 m (depth) by 1 km (length) block. Further details of the acoustic methods we employed are given in Rose and Leggett (1988b,c).

During each transect, we made two types of sea temperature measurements. During 1985, near surface temperature was measured periodically with a mercury thermometer. During 1986, near surface (2 m depth) temperature was measured continuously and digitized each 50 m with a Ryan Tempmentor thermistor (Ryan Corp., WA) wired into the hydroacoustic towed body. During both 1985 and 1986, an average of three expendable bathythermograph (XBT Sippican Corp., Mass) temperature profiles were taken daily. The number of profiles taken daily reflected the variability encountered in near surface temperature. A maximum of eight profiles was taken when variability was high. In both 1985 and 1986, additional temperature information was provided by six Aanderaa recording meters and 10 Ryan recording thermographs deployed at varying depths across the study area (Rose and Leggett 1988b, 1989a).

A sea temperature was assigned to each 10 m (depth) by 1 km (length) block. These were interpolated from temperatures derived from XBT profiles, near surface serial measurements, and deployed instruments. Within block variance in horizontal temperatures, was, in general, low (average SE <0.1°C). Transects which crossed fronts were exceptional. These displayed much higher variability (the maximum change in near surface temperature within a 1 km block was 2.1°C). Transects which crossed fronts comprised less than 2% of transects. Nevertheless, fish were at times aggregated near fronts (Rose and Leggett 1989b), and the averaging necessitated by the scale of our blocks may have introduced some error in the relationship between fish distribution and temperature. This error is thought to be small, however, because the temperature group ranges we used in the more detailed daily analyses were relatively large (2°C). In addition, the aggregation dimensions of both cod and capelin were, in most cases, greater than the block scale (1 km) (Rose and Leggett 1989b). Hence, any errors in temperature assignments were confined to only a fraction of aggregations occurring near fronts. The vertical accuracy of the interpolation was tested by unscheduled XBT profiles taken at random locations. Observed and interpolated temperatures were then compared. The major interpolation error occurred at mid-depths and was due to variations in the thermocline profile. Fortuitously,

mid-depth waters contained few fish. Capelin tended to be most common in surface waters and cod in bottom waters. Near surface and near bottom temperature interpolations were found to be accurate within ±1°C.

For seasonal analyses, transect blocks were assigned to temperature strata grouped over 1°C intervals (i.e., −0.5 to 0.5, 0.5 to 1.5. . .). From these data, the occurrence of each species (presence or absence of acoustic signal equivalent to more than 1 fish) was calculated for the range of temperatures encountered. To compare occurrences among temperature groups having different n, an index of occurrence was used: (observed occurrence − expected occurrence)/expected occurrence, where expected occurrence was proportional to sampling effort.

For daily analyses, sample sizes precluded 1°C intervals. Temperatures were therefore grouped by 2°C intervals. The lowest daily temperature grouping we used was centered on −2°C. In reality this group included only temperatures above −1.7°C, the minimum observed.

We used standard correlation and regression techniques to compare the proportions of the daily abundances of cod and capelin occurring in each 2°C temperature strata. Proportions covered a wide range of values, and were therefore subjected to angular sine transformation prior to analyses (Steel and Torrie 1960).

Statistical analyses employed SAS/STAT for personal computers (SAS Institute, Cary, NC), and STATGRAPHICS (Statistical Graphics Corp., Rockville, MD). Statistical significance is denoted if $P < 0.05$.

Results

A total of 3 181 and 11 575 blocks (1000 by 10 m) were sampled in the summers of 1985 and 1986, respectively, over a range of temperatures of −1.7 to 13.5°C (Fig. 2a and b). Cod occurred in 490 blocks (15%) in 1985 and 1 433 blocks (12%) in 1986. Capelin occurred in 578 blocks (18%) in 1985, and 3 668 blocks (32%) in 1986.

Cod occurrences relative to temperature were similar and nonrandom in 1985 and 1986 (χ^2-tests) (Fig. 2c and d). In 1985, cod occurrence was greater than expected relative to sampling effort at temperatures between −0.5 and 4.5°C, and between 6.5 and 7.5°C. In 1986, cod occurrence was greater than expected at temperatures between 0.5 and 5.5°C. At more extreme temperatures, cod were encountered less often than expected.

Capelin occurrences were also nonrandom relative to temperature in 1985 and 1986 (χ^2-tests) (Fig. 2c and d). However, unlike cod distributions, capelin aggregated at very different temperatures in the 2 yr studied. Capelin occurrences were greater than expected between 5.5 and 10.5°C in 1985, and in waters having even higher temperatures. In 1986, capelin occurrences peaked at much lower temperatures, between −1.5 and −0.5°C and 2.5 and 5.5°C.

More than 95% of the cod surveyed were located at temperatures between −0.5 and 8.5°C in both 1985 and 1986 (Fig. 2e and f). By contrast, capelin distributions relative to temperature differed between years. In 1985, more than 90% of capelin were surveyed at temperatures within the range 1.5 to 8.5°C. In 1986, nearly 40% of capelin were located at temperatures below 1.5°C, but as in 1985, only low densities of capelin occurred at temperatures greater than 8.5°C.

The maximum densities of cod occurred at the same temperatures as the maximum densities of capelin, but only on days when capelin densities were relatively high (Fig. 3). For example, in both years when mean capelin densities exceeded 100 fish/10^5m^3, cod were concentrated where temperatures were similar to those at which capelin densities were maximum (i.e. July 15th–19th, 1985; June 24th–29th, July 8th–11th, 1986). During periods of very high mean capelin density (>400 fish/10^5m^3), cod and capelin aggregations were virtually identical relative to sea temperature (July 17th–18th, 1985; June 25th–26th and July 8th–9th, 1986). These relationships between capelin densities and cod distributions held only at temperatures within the range 0 to 8°C. When capelin were aggregated at more extreme temperatures, cod distributions appeared to be unrelated to those of capelin (i.e., July 23rd, 1985; June 30th and July 4th, 1986).

By contrast, in both 1985 and 1986, cod were consistently aggregated at temperatures between 0 and 5°C when mean capelin densities were relatively low (e.g. <10 fish/10^5m^3) (Fig. 3). This occurred towards the end of July, when capelin were dispersing and dying after spawning, and also on several days during the spawning season (July 22nd, 1985; July 5th, 7th, and 12th, 1986).

To assess the daily correlation between cod and capelin distributions, under conditions of low and high capelin densities, we calculated the daily proportions of each species that occupied each available temperature strata. Each day of 1985 and 1986 was classified with respect to mean capelin density: low densities were those below 100 capelin/10^5m^3; high densities were greater. Initial comparisons indicated that under both density conditions, between year differences in regression slopes were not significant. Hence, data were pooled for further analyses.

Cod densities "tracked" those of capelin relatively poorly under conditions of low capelin density (Fig. 4a). Cod and capelin densities under these conditions were only weakly correlated ($r = 0.30$, df = 145), and the slope of the regression (0.3, SE = 0.1) was only marginally greater than zero. By contrast, cod densities "tracked" capelin densities much more closely under conditions of high capelin density (Fig. 4b). At these times cod and capelin densities were strongly positively correlated ($r = 0.61$, df = 108) and the slope of the regression (0.6, SE = 0.1) was significantly greater than zero (t-test).

We tested the consistency of the relationship between cod and high capelin densities over the range of temperatures surveyed. Cod and capelin densities were significantly and positively correlated within temperature strata centred on 2 to 8°C (range 1–9°C) (Table 1). Regression slopes averaged 0.6 and did not differ significantly among strata. At the more extreme temperatures, there was no significant correlation between cod and capelin densities.

Finally, we tested for between year differences in the subset of our data for which cod-capelin densities were most closely correlated (high capelin densities, 1–9°C). In both years the regression was significant and positive. In 1986 the slope of the relationship ($r = 0.66$, slope = 0.7, SE = 0.1, df = 71) was greater than that observed in 1985 (r) = 0.40, slope = 0.5, SE = 0.2, df = 14), but this difference was not significant (t-test). Pooling of the 1985 and 1986 data also yielded a significant and positive regression ($r = 0.64$, slope = 0.7, and SE = 0.1, df = 86) (Fig. 4c).

Discussion

The data supported our hypothesis that under conditions of low prey density, cod of commercial size (>45 cm) would

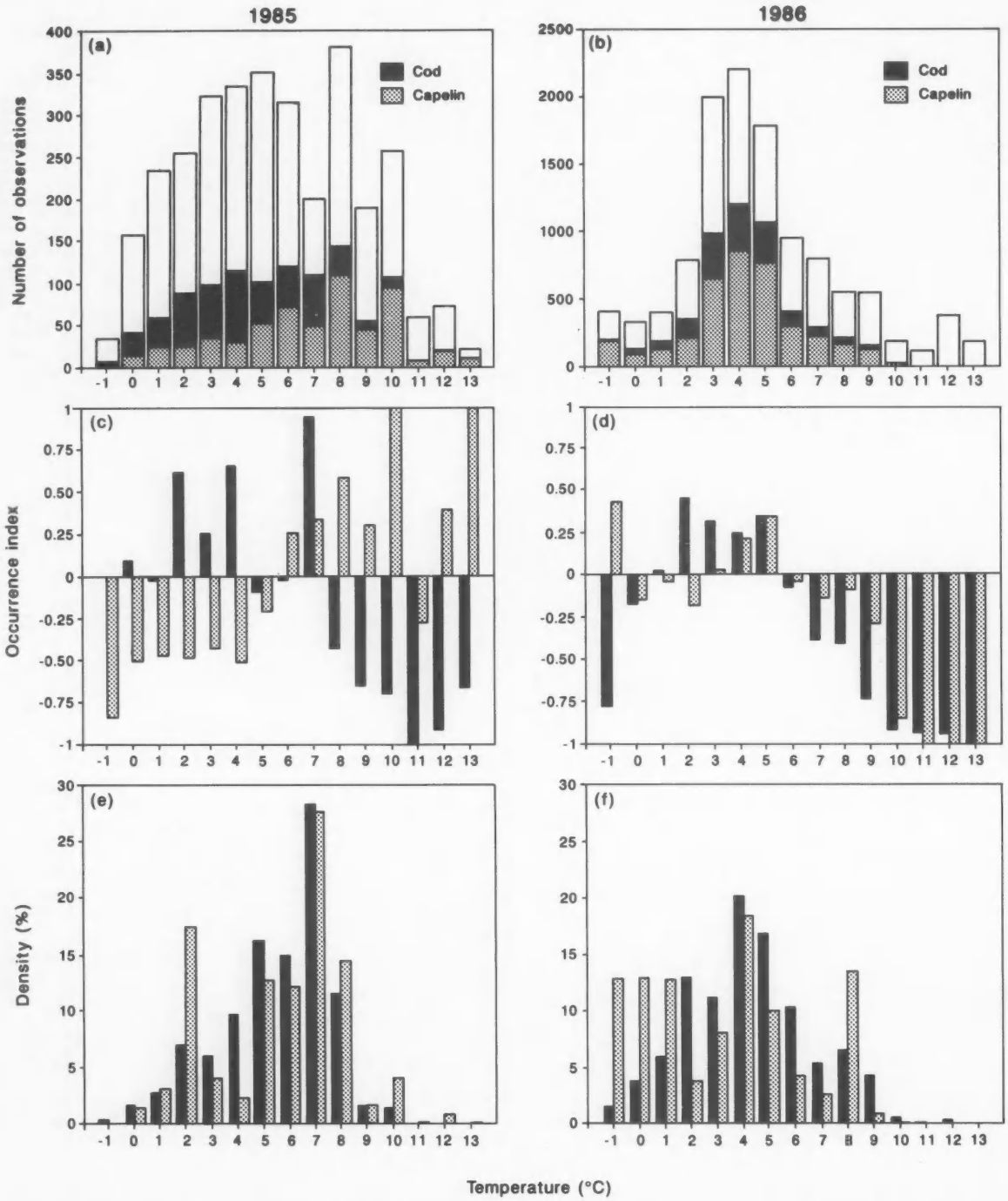

FIG. 2. Distributions of cod (black) and capelin (stippled), as determined acoustically, over the range of sea temperatures encountered during 1985 and 1986 at Brador Bay. (a) and (b) Numbers of observations superimposed on total *n*. (c) and (d) Indices of occurrence (values are ((observed − expected) / expected)) − expected values assume fish distribution to be proportional to sampling effort. For 1985, observed values differed significantly from those expected for both cod and capelin ($\chi^2 = 265$ for cod, 167 for capelin, 13 df, n<5 grouped). This also held for 1986 values ($\chi^2 = 1347$ for cod, 400 for capelin, 11 df). Negligible signals (equivalent to <1 fish/$10^5 m^3$) were discounted. (e) and (f) Distributions of densities (% of total for each year).

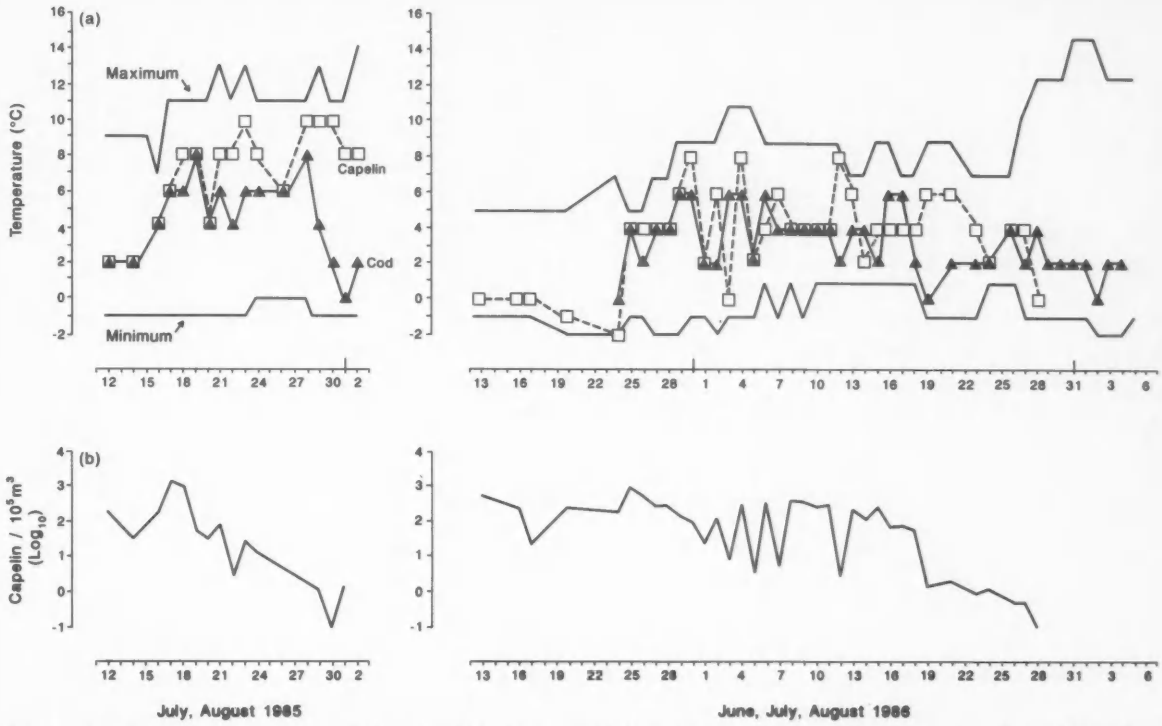

FIG. 3. (a) Daily temperatures at which densities of cod (black) and capelin (open) were greatest in relation to sampling effort at Brador Bay during 1985 and 1986. Ranges of temperatures surveyed daily are outlined. (b) Mean densities of capelin (note log scale) surveyed daily at Brador Bay during 1985 and 1986 as determined by hydroacoustic integration scaled by an estimate of the mean backscattering cross-section for this species (0.0003576 m^2; Rose and Leggett 1988a).

occupy waters having sea temperatures within the modal range (0–5°C) for this species. This range was consistent both between and within years, regardless of the range of available temperatures. For example, when capelin densities declined at the end of July in both 1985 and 1986, cod did not congregate at temperatures where the remaining capelin were found. Rather, they retreated to waters having 0–5°C temperatures. This 0–5°C range is consistent with many reports of temperatures at which commercial sized cod are most frequently lcoated (Hachey et al. 1954; Rasmussen 1954; Martin and Rojo 1955; Bratberg 1960; Jean 1964; Templeman and May 1965; Midtunn 1965; Nakken and Raknes 1987; Rose and Leggett 1988b; Wells et al. 1988). We know of no experimental studies that have determined the "preferred" temperature range (sensu Fry 1947) for cod. Hence, comparisons of preferred and modal temperatures are not possible. However, the final preferendum of this species has been reported to be 1–2°C (Tatyankin 1972), which falls near the center of the modal range we determined.

Our data were also consistent with many reports that cod occasionally occurred in large numbers at temperatures well outside the modal range 0–5°C (Sleggs 1932; Templeman 1954; Jean 1964; Woodhead and Woodhead 1965; Templeman and May 1965; Rose and Leggett 1988b; Wells et al. 1988). These data and our own lead us to conclude that in the Northwest Atlantic cod occur only infrequently in waters having temperatures outside the range −0.5 to 8.5°C. This range may differ somewhat among stocks (e.g. Thompson 1943). Southern races of cod have been reported to frequent temperatures as high as 12°C (Bigelow and Schroeder 1953). It is not known whether such differences are genetically based or result from acclimation to differing oceanic regimes.

Occurrences of North Atlantic cod at temperatures outside the range −0.5 to 8.5°C are not unknown. For example, apparently healthy large cod have been found at temperatures of −1°C on the Newfoundland Grand Banks (H. Lear and C. Bishop, Fisheries and Oceans, St. John's, personal communications), and at Spitzbergen (Beverton and Lee 1965). However, these occurrences appear to be exceptional and to involve the production of blood antifreeze compounds, an acclimation process for which the cod is only poorly adapted.

Laboratory studies have shown that cod can withstand temperatures as low as −1.3°C if adequately acclimated (Dannevig 1930; McKenzie 1934; Harden-Jones and Scholes 1974). However, cod require several weeks of acclimation at temperatures near 0°C to produce the antifreeze compounds necessary to withstand temperatures much below −0.5°C, (Hew et al. 1981; Fletcher et al. 1987). In accordance with this experimental evidence, massive cod mortalities are known to have occurred at these temperatures, presumably when temperature declines from which the fish could not retreat occurred too quickly for acclimation to take place (e.g., Thompson 1943; Templeman and Fleming 1965).

Size, age, and sexual condition may affect acclimation rates to cold temperatures (e.g. Kao and Fletcher 1988). Most of the cod studied by Harden-Jones and Scholes (1974) and observed by Templeman and Fleming (1965), at temperatures of −1°C,

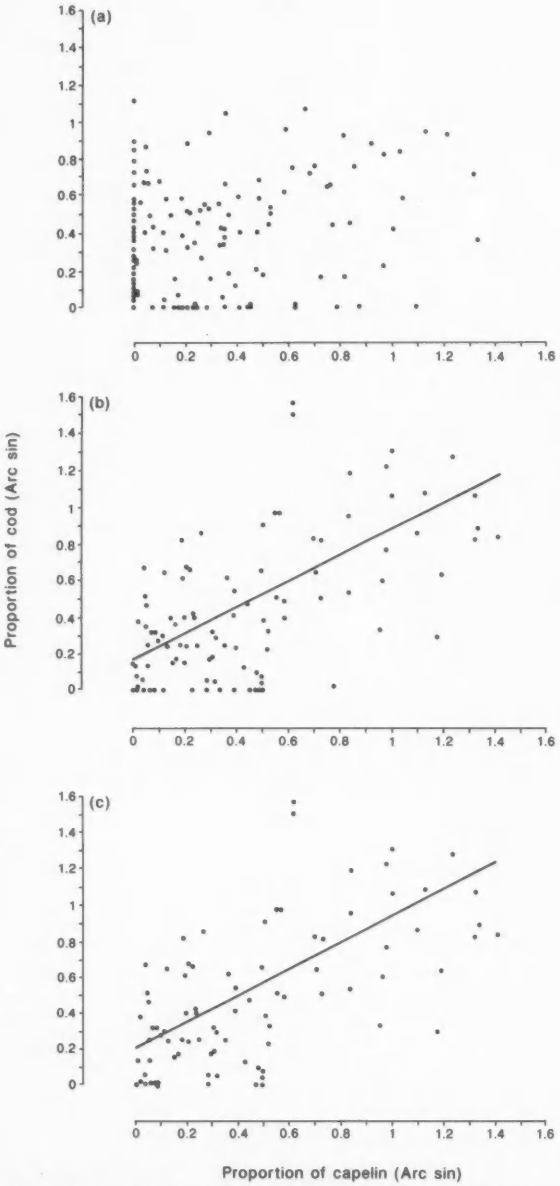

FIG. 4. Proportions of cod relative to those capelin at Brador Bay during 1985 and 1986: (a) at all observed temperatures when mean capelin densities were below $100/10^5m^3$ ($r = 0.30$, slope = 0.3, SE = 0.1, $P < 0.05$); (b) at all temperatures when mean capelin densities were high ($>100/10^5m^3$) ($r = 0.61$, slope = 0.6, SE = 0.1, df = 108, $P < 0.05$); and (c) at temperatures between 1 and 9°C when mean capelin densities were high ($r = 0.64$, slope = 0.7, SE = 0.1, df = 86, $P < 0.05$). Values plotted are angular sine transformations of the daily proportions of cod and capelin found at the same temperatures.

were small and immature (< 45 cm). Small cod appear to be capable of enduring temperatures well below those tolerated by larger cod (Bigelow and Schroeder 1953; Templeman and Fleming 1965). On the north shore of the Gulf of St. Lawrence, small cod (< 30 cm) are relatively common in waters having

TABLE 1. Relationships between proportions of cod and capelin occupying temperature strata encountered at Brador Bay in 1985 and 1986. Values were daily percentages of densities of each species occurring in each temperature strata, and were subjected to angular sine transformation prior to analysis. Temperatures with n <5 not reported. r = pearson correlation coefficient, b = slope, na = not applicable, * = P<0.05.

	Sea temperature (°C)					
	0	2	4	6	8	10
r	<0.01	0.52*	0.52*	0.45*	0.77*	<0.01
b	na	0.74	0.52	0.49	0.53	na
n	14	24	24	23	16	5

sub-zero (to −1.5°C) temperatures (L. Dickie and G. A. Rose, unpublished data). By contrast, larger cod have been reported to die when subjected to temperatures below −1°C (Thompson 1943; Templeman and Fleming 1965). These observations are consistent with experimental evidence that younger and smaller cod are able to produce double the antifreeze levels of larger cod (> 45 cm) and hence are capable of withstanding much colder temperatures (Kao and Fletcher 1988).

It has been hypothesized that cod may penetrate or traverse waters having temperatures below −0.5°C during daily or seasonal migrations (Lear et al. 1986). The extent to which they actually do so is not known. Varying degrees of pre-exposure to low temperatures, both spatially and temporally, may account in part for variable responses of migratory cod to extreme temperatures (e.g., Thompson 1943).

Cod may also acclimate to higher temperatures. Cod have been observed at temperatures as high as 12 and 14.5°C in the North Sea (Woodhead and Woodhead 1965), 12°C in the Gulf of St. Lawrence (Thompson 1943; Rose and Leggett 1988c), and 15°C on the Nantucket Shoals, the most southerly year-round cod ground (Bigelow and Schroeder 1953). McKenzie (1934) reported experimental work indicating acclimated cod could withstand temperatures above 20°C. The rates, limits, and physiological basis of acclimation of cod to warm waters are not known.

Our data indicate that the occurrence of cod at temperatures outside the modal range results from their response to the distribution of abundant prey. In other studies, mature cod have been observed at temperatures near −1°C when in proximity to distributions of capelin occupying even colder waters (Lilly 1982; L. Dickie and G. A. Rose, unpublished data).

Capelin occurred frequently and in abundance at temperatures between 4 and 8°C in both 1985 and 1986. However, capelin occurred over a wider and more variable range of temperatures than did cod. Capelin occasionally occurred in great abundance at temperatures as low as −1.5°C and as high as 10°C. These ranges and variability appear to be characteristic of capelin during the onshore migratory period (B.S. Nakashima, Department of Fisheries and Oceans Canada, St. John's, NF, personal communication). However, in both 1985 and 1986, capelin beach spawning activity in our study area was most intense when nearshore temperatures were between 4 and 8°C. This range is typical of temperatures at which capelin spawn (Templeman 1948).

Our data were consistent with the hypothesis that at mesoscales (10's to 100's of km), and under conditions of high prey density, cod distributions would be positively correlated with those of their prey. However, this occurred only within waters

having temperatures within the 1–9°C range. At temperatures beyond these limits, cod and capelin densities were not related. For example, capelin densities were high ($> 100/10^4m^5$) in waters having temperatures well above 9°C during mid-July, 1985, but no cod were found there, even though cod were abundant elsewhere at temperatures closer to the modal range. During June 1986, capelin densities were very high ($>10000/10^5m^3$) at temperatures < -1°C, but cod were absent there. Analyses of cod stomachs collected during these periods indicated that cod were feeding heavily on capelin (our unpublished data). We cannot, therefore, exclude the possibility that cod moved into waters having extreme temperatures for brief periods during evening feeding forays. It is clear, however, that they did not remain there long, as cod were only rarely encountered at these temperatures. It is also possible that these cod had been feeding on the less abundant capelin that occurred in waters having less extreme temperatures.

The distributions of low capelin densities had no apparent influence on cod distributions. We note that the threshold capelin density above which cod distributions relate to those of capelin, is a mean, and not a maximum, value. Maximum daily capelin densities remained much more constant throughout this study than did mean densities, and cod distributions were not consistently related to them.

Under conditions of high prey density and favorable temperatures, cod distributions "tracked" those of capelin closely. The proportionality of cod and capelin densities at mesoscales may be expected to be even higher during the evening, when cod actively pursue capelin (Dickson 1988; Rose and Leggett 1989b). These findings suggest that foraging by cod on capelin maximizes the feeding potential of this predator, in keeping with "ideal distribution" theory (Fretwell and Lucas 1970). According to this theory, equal average access to food occurs when predator and prey abundances are distributed proportionally (i.e., slope = 1). We found cod and capelin densities to be significantly and positively correlated. However, the slope of the regression of predator onto prey densities was significantly <1 in both 1985 and 1986. Other workers have reported similar results for other species (e.g., Hassell and May 1973; Sutherland 1983; Gillis and Kramer 1987).

The slope of the regression of predator onto prey densities may differ from unity for many reasons. Conspecific predators may interfere with each other as their densities increase. This may result from aggression by the largest or most dominant predators (Fretwell and Lucas 1970), non-aggressive reductions in foraging efficiencies (Gillis and Kramer 1987), or from a variety of density-dependent causes (Eggers 1976; Ens and Goss-Custard 1984). We can only speculate on the causes of this result in our case. The schooling behavior of capelin may cause prey "catchability," as perceived by the predator, to differ from that indicated by our measures of density. For example, a given quantity of capelin aggregated into a small number of large schools has a much smaller surface area for attack than does the same quantity of capelin organized into numerous smaller schools. We observed that capelin schools were typically larger where capelin abundance was higher, and that cod attacked capelin from below and at the circumference of the schools. If predator distributions reflect responses to prey catchability, rather than to absolute prey density, a slope of <1 would be expected given the schooling behavior exhibited by capelin.

The conclusion that prey distributions partly regulate cod distributions at mesoscales in the nearshore zone indicates that variability in capelin distribution patterns, whether caused by natural events or by harvesting, will have a direct impact on the availability of cod to the inshore fixed-gear fisheries.

The success of commercial fisheries for cod depends on the local occurrence of high fish densities. Our data clearly indicate that these high densities occur most commonly at locations where temperatures are within the -0.5 to 8.5°C temperature range. Extensive data from several years of spring bottom-trawl surveys off Newfoundland indicated that large catches of cod are uncommon where bottom temperatures are < -0.5°C (Wells et al. 1988). Hence, successful exploitation is likely to occur at temperatures outside the range -0.5 to 8.5°C only under exceptional circumstances. In the absence of acoustic information on cod distributions, fishing is likely to be most successful in waters having temperatures near 2.5°C, the midpoint of the modal range for this species (0–5°C). The exceptions to these generalizations will occur when prey densities are high at temperatures outside the modal range. We have demonstrated that cod will aggregate, at least temporarily, in waters having temperatures well outside the 0 to 5°C range under these conditions.

It is clear that neither sea temperature nor prey density alone provide consistent explanations of cod distribution or catch. Reports of "best" catches of cod over narrow temperature ranges can, in virtually every case, be ascribed to short term and small scale observations (when any tolerable temperature could be described as "best"), and by the lack of consideration of other factors (geophysical and biotic), which affect cod distribution (e.g. Sleggs 1932; Thompson 1943). In a separate study, we found that the explanatory power of temperature in accounting for daily variations in nearshore cod trap catch rates in 1985 was limited to setting the temperature limits (-0.5 to 8.5°C) outside of which no cod would be caught (Rose and Leggett 1988b). These conclusions show clearly why several previous studies have been unable to demonstrate a clear relationship between sea temperature and catch rates (Akenhead et al. 1982; Buchanan et al. 1982; Lear et al. 1986; Aggett et al. 1987).

We believe the conclusion that sea temperature (directly and as a proxy for currents and salinities) and prey density interactively regulate cod distribution is applicable to all cod populations. For example, cod distribution has been reported to be related to food abundance where hydrographic conditions are tolerable off Greenland (Hansen 1949), in the Northwest Atlantic (Hachey et al. 1954), and off Northeastern Newfoundland and Labrador (Templeman 1966). For Barents Sea cod, Beverton and Lee (1965) reported that food abundance was not a major factor in determining their distribution at large scales (hydrographics were attributed to be the major factor), but that "the presence of a restricted concentration of a preferred food . . . may result in significant aggregations of cod . . . within a region which is hydrographically tolerable." These results are not expected to apply during reproductive or other non-feeding periods.

Neill and Magnuson (1974) provide experimental evidence that our results may also apply to other species. In their study of bluegill (*Lepomis macrochirus*) and perch (*Perca flavescens*) these fish foraged at their long-term preferred temperatures when food was abundant there, but actively selected temperatures outside this range when food was more abundant at these extreme temperatures. Perch and bluegills also exhibited species specific minimum and maximum temperatures beyond which they would not venture to feed regardless of prey dens-

ities. Neill and Magnuson (1974) also argued that the heterogenous distribution of fishes in a Wisconsin lake primarily reflected longer term temperature preferences. However, at shorter time scales, significant deviations from expectation occurred as fish exploited locally abundant food supplies.

We conclude that the mesoscale patterns of cod distribution observed in the northeastern Gulf of St. Lawrence resulted from the interactive responses of this predator to both geophysically-forced sea temperatures and prey densities. The specific levels of each factor to which the cod responded appeared to be constant over two summers of observation. It is clear that quantification of both geophysical and biotic factors is necessary to provide consistent accounts of the distribution patterns of this, and likely most other, species.

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Microbiological Characteristics of White Whale (*Delphinapterus leucas*) From Capture Through Extended Captivity

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For proper husbandry, it is important to sample the environment from which marine mammals for captivity are taken and to monitor the animals frequently over an extended period. Samples for microbiological analysis were collected from 20 beluga whales during capture in the Churchill River. Seven animals were retained in three U.S. aquaria with anal and blowhole cultures taken over a 945-d period. Bacteria isolated from whales at capture included many soil and water forms found also in environmental samples. *Pasteurella multocida* was isolated from three whales at capture and infrequently several times in captivity from two of these which were studied the most intensively. From this latter group maintained at Mystic (CT) Marinelife Aquarium, the most frequently recovered bacteria from both animals were members of the *Achromobacter-Acinetobacter-Alcaligenes*-CDC IV E-*Moraxella-Pasteurella-Pseudomonas* group, *Escherichia coli, Pseudomonas putrefaciens, Staphylococcus epidermidis, Streptococcus faecalis*, and *Vibrio fluvialis*. Only one of these whales yielded cultures of *Klebsiella pneumoniae, Plesiomonas shigelloides*, and coagulase positive *Staphylococcus aureus*. Species of *Acinetobacter, Aeromonas, Pseudomonas*, and *Vibrio* were most common in whales maintained at the other aquaria although, as noted for animals at Mystic, several species were isolated from some samples and were absent in others.

Pour élever correctement des bélugas en captivité, il est important de prélever des échantillons dans le milieu d'où proviennent les animaux et de procéder fréquemment à l'examen de ceux-ci durant une longue période. Nous avons prélevé des échantillons sur 20 bélugas pour fins d'analyses microbiologiques au moment de leur capture dans le fleuve Churchill. Sept baleines ont été gardées en captivité dans trois aquariums américains et des cultures bactériennes d'échantillons prélevés au niveau de l'anus et de l'évent ont été réalisées sur une période de 945 jours. Plusieurs formes bactériennes prélevées sur les bélugas au moment de la capture étaient aussi présentes dans les échantillons d'eau et de sol recueillis dans l'environnement. On a retrouvé *Pasteurella multocida* chez trois baleines au moment de la capture et, durant la captivité, nous avons également détecté à quelques reprises, quoique assez rarement, cette bactérie chez les deux bélugas qui ont été suivies le plus intensivement. Chez ces deux bélugas gardées au Marinelife Aquarium de Mystic (CT), les bactéries les plus fréquemment isolées appartenaient au groupe des *Achromobacter-Acinetobacter-Alcaligenes*-CDC IV E-*Moraxella-Pasteurella-Pseudomonas*, où étaient des *Escherichia coli, Pseudomonas putrefaciens, Staphylococcus epidermidis, Streptococcus faecalis* ou *Vibrio fluvialis*. Un seul des bélugas a donné des cultures de *K. pneumoniae, Plesiomonas shigelloides* et *Staphylococcus aureus* coagulase-positif. Certaines espèces d'*Acinetobacter*, d'*Aeromonas*, de *Pseudomonas* et de *Vibrio* ont été retrouvées plus fréquemment chez les bélugas gardées dans les autres aquariums, mais, comme dans le cas des animaux gardés à Mystic, plusieurs espèces de bactéries se retrouvaient dans certains échantillons et pas dans d'autres.

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The white whale (*Delphinapterus leucas*) is found commonly in the waters of Hudson Bay (Doan and Douglas 1953; Sergeant 1973; Hay and McClung 1976; Smith and Hammill 1986) and most individuals now in captivity are from the western Hudson's Bay population. Some information is available on microbial disease in live (Dunn et al. 1982) and dead (MacNeill et al. 1978) belugas in captivity and in dead stranded animals (Martineau et al. 1988), but there are no data

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on microorganisms living in the wild. This is important because proper husbandry should include knowledge of the normal microbial flora. While capturing belugas in the Churchill River during the summers of 1985 and 1987, we performed microbiological studies on both the waters from which the whales were taken and the animals themselves. A total of 20 whales was captured, of which 13 were released immediately. Two are currently maintained at the National Aquarium in Baltimore, three are at the New York Aquarium, and two are located at Mystic Marinelife Aquarium in Connecticut. Studies have continued on the last group for approximately 19 mo and are the major subject of this report.

Our purpose was to establish baseline information on naturally-occurring microbes in wild white whales and their environment so that changes in the microflora of captive animals could be assessed. Although these data may not apply to all belugas in captivity, they nonetheless provide a useful model.

Materials and Methods

River Water

Water samples were taken from the Churchill River in the capture area (Fig. 1) on the western shore of Hudson's Bay in Manitoba, Canada. In July 1985 subsurface (approximately 10 cm) and deeper samples (Table 1) were collected using a sterile bag device (Niskin 1962) and processed in the laboratory within 2 h. In 1987 only subsurface water was sampled. Membrane filtration (0.45 μm membranes) platings were made on sheep blood agar, eosin methylene blue agar (EMB), mannitol salt agar, and Sabouraud dextrose agar containing 150 mg/L of chloramphenicol (SD^+). All plates were incubated at 35°C for 1–3 days. Representatives of all colony types were picked and streaked to ensure purity. The Gram reaction (Buck 1982) and cytochrome oxidase test (Pathotec Strips; General Diagnostics) were recorded for each isolate. Gram negative bacteria were identified using the API 20E system (Analytab Products). The STAPH Trac and Rapid STREP systems (both Analytab Products) were used for Gram positive cocci. In 1985 water was passed through 0.45 μm membranes and cultured on m-Endo broth and m-FC broth for enumeration of total and fecal coliforms, respectively.

Whale Capture and Sampling

Beluga whales are abundant in the Churchill River during the late summer. Native Inuits in small boats herded whales from the area of Sta. 5 and 6 (Fig. 1) into water approximately 1 m deep, where the animals were surrounded and carefully restrained. Swabs (Culturette II; Marion Scientific Corp.) were inserted into the blowhole to a depth of several cm. In 1985 a total of 10 whales was sampled; six were released immediately and four were retained for shipment to Connecticut and New York. Ten whales were also captured in 1987; seven were released and three were kept for shipment to New York. Swabs were processed in the laboratory within 2 h. Plates of blood, EMB, mannitol salt, and SD^+ agar were divided into three sections in a "T" form. The upper sections were inoculated directly with the swabs and the remainder of the plates were streaked serially with a sterile wire loop to obtain isolated colonies. The tips of the swabs were broken off in tubes of trypticase-soy broth and SD^+ broth. All plates and tubes were incubated at 35°C for 24–28 h. Representative colonies were selected, purified, and identified as described above.

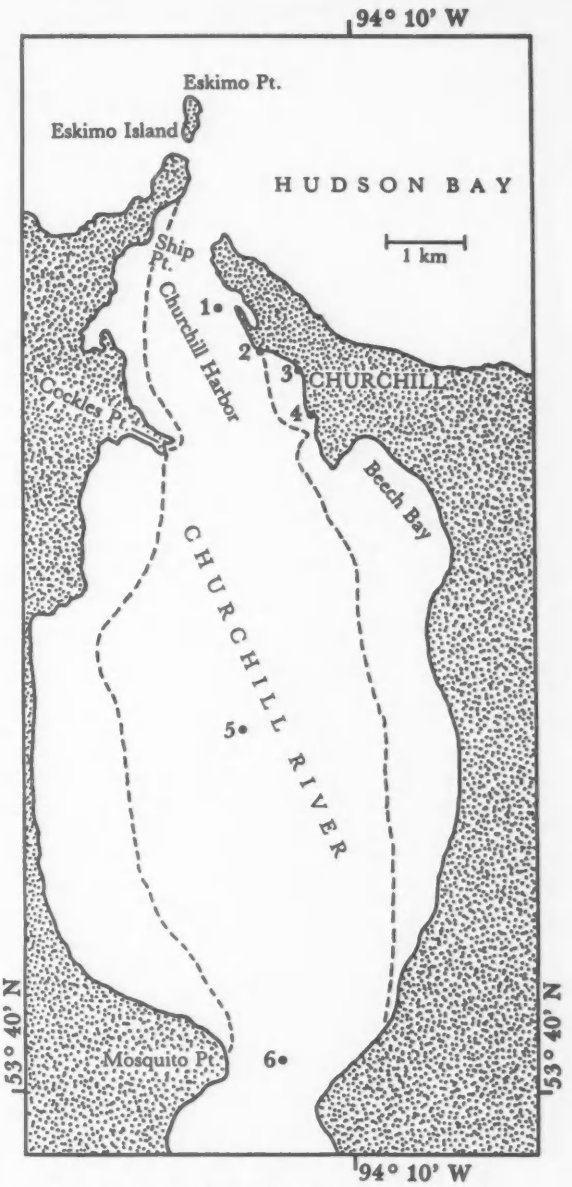

FIG. 1. Sampling Sites in the Churchill River, Canada.

Whale Transport

The seven belugas retained for subsequent shipment in both years were placed in stretchers, moved to shore, and released into a holding pool containing recirculated Churchill River water. They were in the pool for 3–6 days with white blood cell counts monitored daily. Individual open boxes were constructed to support each whale in its stretcher for final shipment. The boxes were lined with polyurethane foam to absorb shock and minimize contact of the whales with hard surfaces or waste products. Each animal was coated with vegetable shortening to prevent skin dehydration, and ophthalmic ointment was applied to the eyes. Muslin sheets were draped over the whales and

TABLE 1. Membrane filter coliform counts in the Churchill River in the area of whale capture.

Date and location	Tide	Depth (m)	Salinity (‰)	Temperature (°C)	No./100 mL Total coliforms	No./100 mL Fecal coliforms
24 July 85						
Sta. 2	Ebbing	2	30.8	4	5	2
Sta. 4	Ebbing	0 (surface)	30.6	4	13	1
25 July 85						
Sta. 1	Low	0	8.5	11	52	11
		7	17	10	65	10
	High	1	28.1	8	7	<1
		7	31.2	5	3	<1
Sta. 2	High	7	32.6	4	10	<1
Sta. 5	Low	0	2.5	14	20	2
		4.5	5.5	12	22	11
Sta. 6	Ebbing	1	2.4	14	55	3
28 July 85						
Sta. 1	High	0	25.9	°ç±	1	2
		7	31.0	8	30	<1
Sta. 5	Ebbing	0	2.8	15	1	2
		4.5	28.5	10	6	<1

moistened with water; ice was packed around the dorsal surface, flippers, and flukes. Temperature inside the chartered jet aircraft was maintained at 6°C. During the flight the animals were misted with water, respiration rates recorded, and blood samples collected.

On arrival in New York City in 1985, two belugas were offloaded into a refrigerated truck and driven to Mystic Marinelife Aquarium. The other two animals were transported to New York Aquarium and maintained until subsequent shipment to Baltimore. In 1987 all three whales were kept at New York Aquarium which obtains water from a seawater aquifer.

At Mystic the boxes were unloaded and the stretchered whales were placed in a freshwater holding pool. The animals spent a total of 11.5 h out of water during transit. Quarantine was maintained for eight days with freshwater and saltwater being alternated in the pool to prevent dehydration and facilitate removal of external parasites. Subsequently, normal aquarium water was used in the pool (Dunn et al. 1982).

Microbiological Sampling in Captivity

Frequent swab samples were obtained from both anus and blowhole of each whale during routine physical examinations. Platings, isolations, and microbial identifications were conducted as above; the API Yeast-Ident system (Analytab Products) was added for yeast identification. In addition, blowhole bacteria were isolated from animals at Mystic by exposing plates to blowhole aerosols. The belugas were conditioned to expel air from the blowhole on command, allowing plates to be exposed to this "mist".

Results and Discussion

Total and fecal coliform counts in the area of beluga capture were low at varying depths and hydrographic conditions (Table 1) indicating that at the approximate time of whale capture there was no evidence of heavy domestic pollution of the Churchill River. The town operates a small sewage treatment plant near the capture site. Effluent enters the river above the capture area (between Sta. 2 and 4), but at ebbing or low tide coliform counts at these locations were not substantially different than at other stations. We concluded that either the volume of sewage treated was small relative to the river volume and subsequent dilution was great, the plant was effective in reducing coliform densities, or coliforms discharged from the plant did not survive the saline environment of the river.

Several organisms were common in both years in river water (Table 2), and many are soil- or water-associated bacteria of no primary pathogenic significance. However, several are potentially pathogenic in humans (*Aeromonas hydrophila, Candida* spp., *Klebsiella pneumoniae, Vibrio cholerae*). *Vibrio cholerae* has been found in water in which Atlantic bottlenose dolphins (*Tursiops truncatus*) in Hawaii are kept (Schroeder et al. 1985). Martineau et al. (1988) recovered *V. cholerae* from the carcass of a stranded beluga whale in the St. Lawrence Estuary. However, the organism has not been implicated in cetacean disease. *Aeromonas hydrophila* has been found as a secondary invader in manatees (*Trichechus manatus*) in Florida (Irvine et al. 1980), and we recorded deaths of captive Atlantic whiteside dolphins (*Lagenorphynchus acutus*) that may have been caused by *K. pneumoniae* (Buck et al. 1988). Species of *Candida* have also been associated with marine mammal disease (Dunn et al. 1982, 1984).

One sample of sewage treatment plant effluent was obtained in 1987. Isolates included *Acinetobacter calcoaceticus* var. *lwoffi, Acinetobacter-Pasteurella-Pseudomonas* spp., *Candida albicans, C. guilliermondii, C. parapsilosis, C. pseudotropicalis, C. tropicalis, Citrobacter* sp., coryneforms, *Enterobacter cloacae*, and *Proteus inconstans*. Few of these organisms were recovered from river water, probably because of dilution or salinity effects. Several yeasts including potentially pathogenic *Candida* spp. were found in both sewage treatment plant effluent and river water (see below). All isolates can survive in seawater (Hagler and Ahearn 1987).

Some bacteria recovered from the blowhole of 13 temporarily restrained whales (Table 3) are identical or similar to those

TABLE 2. Isolates from membrane filter platings of Churchill River water in the area of whale capture.

1985	1987
Achromobacter-Pseudomonas sp.	*Acinetobacter-Pasturella-Pseudomonas*
Achromobacter-Acinetobacter-Alcaligenes-CDC IV E-*Moraxella-Pasturella-Pseudomonas* sp.	*Aeromonas hydrophila*
Acinetobacter sp.	*Candida albicans*
Aeromonas hydrophila	*C. guilliermondii*
Escherichia coli	*C. parapsilosis*
Fluorescent Pseudomonas sp.	*C. tropicalis*
Hafnia alvei	*Escherichia coli*
Klebsiella pneumoniae	*Klebsiella pneumoniae*
K. oxytoca	*Micrococcus* sp.
Plesiomonas shigelloides	*Vibrio cholerae*
Pseudomonas sp.	
Serratia plymuthica	
Vibrio cholerae	

TABLE 3. Microorganisms recovered from the blowhole of 13 belukha whales captured in the Churchill River and released immediately.

Year and Animal No.	Isolates	Presence in Water
1985		
1	Fluorescent *Pseudomonas* sp.	−
3	*Micrococcus* sp.	−
	Pasteurella multocida	−
6	*Acinetobacter-Achromobacter-Alcaligenes*-CDC IV E-*Moraxella-Pasteurella-Pseudomonas* sp. (AAACMPP)	+
	Acinetobacter-Pasteurella-Pseudomonas sp.	+
	Aeromonas hydrophila	+
	Citrobacter freundii	−
	Fluorescent *Pseudomonas* sp.	+
	Staphylococcus epidermidis	−
	Unidentified Gr-rod	−
7	AAACMPP	+
	Pasteurella multocida	−
8	*Pasteurella multocida*	−
9	*Escherichia coli*	+
1987		
2	*Aeromonas hydrophila*	−
	Fluorescent *Pseudomonas* sp.	+
	Micrococcus sp.	+
3	*Aeromonas hydrolphila*	−
	Pseudomonas sp.	
4	No organisms recovered	
6	*Aeromonas hydrophila*	+
	Bacillus sp.	−
	Pseudomonas sp.	−
7	*Aeromonas hydrophila*	+
	Bacillus sp.	−
	Micrococcus sp.	+
	Pseudomonas aeruginosa	−
8	*Aeromonas hydrophila*	+
	Pseudomonas sp.	−
	Rhodotorula glutinis	−
9	*Aeromonas hydrophila*	+
	Acinetobacter-Alcaligenes-Achromobacter-Pseudomonas sp.	+
	Rhodotorula glutinis	−

found in water (e.g., *Acinetobacter, Achromobacter, Citrobacter, Escherichia, Micrococcus, Moraxella, Pseudomonas*) and were probably indigenous to soil and water. They are likely to be either incidental on the whale blowhole or harmless commensals. Others (e.g., *Staphylococcus* and *Streptococcus* spp.) may be more closely animal-associated and could be secondary invaders in skin lesions or other superficial infections. Of the other isolates, *Pasteurella multocida* was the most significant because of its previous recovery from healthy and diseased lungs of pinnipeds (Geraci et al. 1982; Simpson and Cornell 1983;

TABLE 4. Microorganisms recovered from anal (A) and blowhole (BH) swabs from two belukha whales (#4 and #10) from capture (day 0; 30 July 1985) through day 945 (9 March 1987) of captivity at Mystic Marinelife Aquarium.

Microorganism	Day of captivity when recovered	
	#4	#10
Achromobacter-Pseudomonas sp.	BH 0, 76	
Achromobacter-Acinetobacter-Alcaligenes-CDC IV E-*Moraxella-Pasteurella-Pseudomonas* sp. (AAACMPP)	A 184 BH 0, 184, 361, 466, 510, 566, 648, 656, 670, 707	A 184 BH 24, 51, 76, 184, 411
Acinetobacter calcoaceticus var. *lwoffi*	A 22, 29 BH 29	A 24 BH 670
Acinetobacter calcoaceticus var. *anitratus*	A 24	BH 0
Acinetobacter sp.	A 8	
Acinetobacter-Pasteurella sp.	BH 114	BH 13 BH 76
Aerococcus sp.		
Aeromonas hydrophila	A 8, 22, 41, 51, 184 BH 51, 218, 275, 373,466	A 13, 29 BH 8
Aeromonas salmonicida	BH 945	
Alcaligenes-Pseudomonas sp.	BH 51	
Candida ciferrii	A 29	A 29
Candida guiliermondii	A 22, 24, 29, 41, 51 BH 51	A 24, 29, 51, 53
Candida lambica	BH 83	
Candida pseudotropicalis	A 22, 184	A 24
Chromobacter sp.	BH 0	
Citrobacter freundii	A 17 BH 17	
Corynebacterium sp.	BH 0, 13	BH 114, 139
Enterobacter aerogenes		A 41, 51, 167 BH 184
Enterobacter agglomerans	A 17 BH 17, 83	A 24 BH 17 BH 670
Enterobacter cloacae		
Escherichia coli	A 8, 29, 51, 226, 364, 367, 375, 566, 945 BH 41	A 13, 24, 29, 83, 167, 226, 375, 449 BH 184, 275, 375, 656
Fluorescent *Pseudomonas* sp.	BH 0	BH 0
Klebsiella pneumoniae	BH 51	
Klebsiella sp.	BH 51	
Micrococcus sp.	BH 0, 449, 707, 720	BH 76, 226, 656, 670, 707
Morganella morganii	A 449, 945	A 8, 167 BH 51, 184
Pasteurella multocida	A 41 BH 0, 83, 373	BH 475
Pichia wickerhamii	A 51	A 51
Plesiomonas shigelloides	A 945	
Proteus mirabilis	A 8	A 13, 17, 83, 167, 226, 449 BH 697
Proteus vulgaris	A 8, 226, 364, 367	A 8, 41, 83, 167, 226
Pseudomonas maltophilia	A 22, 29 BH 29, 361	A 24, 29 BH 449
Pseudomonas putrefaciens	A 13, 17, 22, 29, 362, 449 BH 24, 29, 367, 373, 375, 411, 510, 566, 648, 656, 670, 707	A 24, 29 BH 17, 24, 29, 41, 51, 76, 114, 218, 411, 707
Pseudomonas sp.	A 22 BH 90, 648, 707, 720, 945	BH 51, 114, 167, 566, 656, 697
Serratia sp.	A 13	A 17
Staphylococcus aureus (coagulase +)	A 17	
Staphylococcus aureus (coagulase −)	BH 17	BH 76, 566
Staphylococcus cohnii	BH 218	BH 218
Staphylococcus epidermidis	A 17, 184 BH 17, 114, 218, 226, 373, 375, 510, 854	A 184 BH 17, 76, 184, 218, 411, 566
Staphylococcus hemolyticus	BH 275, 854	BH 218
Staphylococcus hominis	A 566 BH 0, 375, 466	BH 218, 275, 411, 566

TABLE 4. (*continued*)

Staphylococcus hyicus	BH 8, 13, 17	BH 8
Staphylococcus saprophyticus	A 17	
	BH 17	
Staphylococcus sciuri	BH 13, 17	
Staphylococcus warneri	A 184	
	BH 0, 17, 184, 854, 945	BH 17, 76
Staphylococcus xylosus	BH 0, 139	BH 0, 411, 475
Streptococcus faecalis	A 24, 29, 51, 167, 375	A 8, 13, 17, 24, 29, 41, 51, 83
	BH 41, 367, 375	BH 17, 375, 449
Streptococcus mitis	BH 139	
Streptococcus sp.	BH 8, 76	BH 707
Torulopsis candida	A 17	
	BH 17	
Trichosporon cutaneum	A 17	
	BH 17	
Vibrio alginolyticus	A 367, 449, 945	
	BH 24, 114, 367, 720, 854	BH 218, 449, 466, 475, 510, 854
Vibrio fluvialis	A 22, 184	A 13, 41, 51
	BH 13, 17, 22, 114, 184, 361, 364, 367, 373, 375, 411	BH 13, 41, 76, 83, 114, 167, 226, 411
Vibrio parahaemolyticus	A 945	
	BH 373, 888	BH 375

Baker 1984). This bacterium is widespread in wild animals including birds, and its presence in river water would not have been unusual. Because the organism was not found in the Churchill River in either year, it may have only been present in small numbers; but its occurrence in three of the six whales in 1985 (Table 3) suggests some degree of adaptation to a whale host. *Pasteurella multocida* is a serious pathogen of both humans and animals (Carter 1981), and recovery from a whale blowhole should be viewed as potentially important. *Pasteurella multocida* was also found at capture in the blowhole of a whale taken to Mystic in 1985. The organism was recovered several times thereafter, but the animal never showed any clinical symptoms of disease. *Pasteurella multocida* was not isolated from any of the animals at capture in 1987 and the organism was not cultured after the whales were placed in captivity. Other potential whale pathogens (e.g., *Klebsiella, Candida*) were found in river water but not in any of the belugas at capture.

A total of 53 different microorganisms or groups of organisms was recovered from the two whales maintained at Mystic (Table 4). Of these, 35 (64%) were found in both animals. The majority of these were recovered either infrequently or were probably not important as potential pathogens. There were only six bacteria found frequently in both whales and included the *Achromobacter-Acinetobacter-Alcaligenes*-CDC IV E-*Moraxella-Pasteurella-Pseudomonas* (AAACMPP) group, *Streptococcus faecalis*, and *Vibrio fluvialis*. The AAACMPP group, *S. epidermidis*, and *Vibrio fluvialis* were most common in blowhole swabs, *E. coli* was dominant in anal samples, and *P. putrefaciens* and *S. faecalis* were recovered often from both anal and blowhole swabs. This pattern of distribution is not considered unusual considering the natural habitats of these bacteria. *Escherichia coli* is found in the intestinal tract of all warm-blooded animals. The AAACMPP group and *V. fluvialis* are common in water and *S. epidermidis* is ubiquitous on the skin of humans and environmental surfaces. The occurrence of these bacteria in the whale blowhole is not inconsistent. *Pseudomonas putrefaciens* and *S. faecalis* are common in feces, soil, and water and their recovery from anal and blowhole swabs is not unusual. With the exception of *V. fluvialis*, the organisms cited above (or closely related ones) that were common in both whales kept at Mystic were found in Churchill River water of the other animals released. However, *V. fluvialis* was found in the whales captured in 1987 (see below and Table 5).

Sixteen of the 53 organisms or groups were found in whale #4 but not in whale #10. The majority of these were infrequent isolates or nonpathogens. However, three species found only in whale #4 are considered potentially dangerous to mammals and included *K. pneumoniae*, *Plesiomonas shigelloides*, and coagulase positive *Staphylococcus aureus*. *Klebsiella pneumoniae* and *P. shigelloides* were isolated from Churchill River water but recovered from animal #4 only after 51 and 945 d, respectively.

Aeromonas hydrophila was recovered frequently from beluga #4. This organism, also found in river water and one other whale at capture, is a serious pathogen of fishes (Austin and Allen-Austin 1985) and humans (Ljugh and Wadstrom 1982–83; George et al. 1985); but there is no evidence that it produces disease in marine mammals. An *Aerococcus* species and *Enterobacter cloacae* were found in only whale #10, and several cultures of *Enterobacter aerogenes* were recovered from this animal. None of these organisms is pathogenic.

Bacterial sampling of two whales maintained at New York Aquarium for acclimation was limited (Table 5); thus, it is difficult to generalize about microbiologic sequence in these belugas except to say that species of *Acinetobacter*, *Pseudomonas*, and *Vibrio* seem to have become a dominant portion of the commensal microflora. The same situation was also observed with whales #4 and #10 maintained at Mystic.

Table 5 also presents data for isolation of bacteria from whales captured in 1987 and subsequently maintained at New York Aquarium. Results for the three whales maintained at New York Aquarium are also somewhat limited compared with the animals at Mystic (Table 4). Generally, all three animals showed a predominance of *Aeromonas hydrophila* and several species of *Pseudomonas* and *Vibrio*. This was not unlike the population seen in the Mystic belugas, and the occasional occurrence (and disappearance) of a large number of other organisms was similar also.

Vibrio cholerae was recovered from the Churchill River in both 1985 and 1987 and from one whale at capture that was

TABLE 5. Bacteria recovered from blowhole swabs from two beluga whales captured in 1985 and three whales captured in 1987 kept in captivity at the National Aquarium in Baltimore and the New York Aquarium, respectively.

	Day of captivity when recovered				
	National Aquarium		New York Aquarium		
Microorganism	#2	#5	87.001	87.005	87.010
Achromobacter-Acinetobacter-Alcaligenes-CDC IV E-*Moraxella-Pasteurella-Pseudomonas* sp.		0		26	
Acinetobacter calcoaceticus var. *lwoffi*	10				
Acinetobacter-Pasteurella sp.	0				26
Acinetobacter-Pasteurella-Pseudomonas sp.	0	0			
Acinetobacter-Pseudomonas sp.			2		
Aeromonas hydrophila			0, 26	0, 26	0
Citrobacter freundii				26	26
CDC IV E		0			
Corynebacterium sp.			3		
Enterobacter agglomerans				82	
Enterobacter cloacae			26	82	
Escherichia coli	0	0			
Flavobacterium-Pseudomonas sp.			26	26	
Fluorescent *Pseudomonas* sp.			3	82	0, 2
Klebsiella Pneumoniae			26		
Klebsiella sp.		331			
Micrococcus sp.	0	0		0	
Pasteurella multocida		0			
Pseudomonas aeruginosa			26	26	
Pseudomonas fluorescens		0			
Pseudomonas maltophilia					26
Pseudomonas paucimobilis	331				
Pseudomonas putrefaciens	231	331			
Pseudomonas sp.			26		0, 2
Serratia marcescens				26	
Staphylococcus aureus (coagulase −)		0			
Staphylococcus xylosus		0			
Staphylococcus sp.					0
Streptococcus faecalis		0			
Vibrio alginolyticus		213	0, 26	26	26
Vibrio cholerae			0		
Vibrio fluvialis			0	26	0
Vibrio sp.	213				

eventually shipped to New York. This bacterium was never isolated thereafter. Other species of *Vibrio* were recovered in disjunct patterns. None was found in Churchill River water in 1985 or 1987, from any of the 13 belugas captured and released, or from the Baltimore or Mystic animals captured in 1985. Two of the New York whales taken in 1987 showed blowhole isolates of *V. fluvialis* at capture and then none in captivity, but *V. alginolyticus* appeared after 3 wk. No vibrios were found in the third New York whale at capture, but both *V. alginolyticus* and *V. fluvialis* were recovered after 3 wk. in captivity. In fact, after capture all seven whales showed the frequent occurrence of *Vibrio* spp. and the disappearance of other bacteria common at capture. Marine vibrios are associated with marine mammals (Buck and Spotte 1986) and were involved in mass mortalities of Atlantic bottlenose dolphins along the east coast of the U.S. during the summer of 1987 (Hersh 1988).

It is difficult to predict what changes will occur, if any, in the microflora of marine mammals after a time in captivity. Differences in water composition (seawater, artificial seawater, brine), water temperature and pH, organic levels, or other variables will influence quantitative and qualitative aspects of animal-associated microorganisms. Nonetheless, we have documented some distinct shifts in the microflora of white whales placed in captivity. As seen in Table 4, whale #4 showed a wide variety of bacteria in both anal and blowhole samples at capture (day 0), some of which were found in Churchill River water. Bacteria recovered later from this animal included several taxa found at capture (e.g., AAACMPP, *A. hydrophila, E. coli, Proteus* spp.) but the population included also routine isolations of *P. putrefaciens, S. epidermidis, S. faecalis, V. alginolyticus,* and *V. fluvialis*. In other words, some species disappeared and others became dominant.

Whale #10 showed only a few species at capture (Table 4, day 0); most were also seen in whale #4. After extended captivity the associated microflora had expanded and included species almost identical with those seen in whale #4. these observations suggested that conditions of captivity influence commensal microorganisms. This is particularly true of the marine vibrios, some of which are known to adsorb to surfaces (Kaneko and Colwell 1978). They occur commonly in captive animals and are potentially transmissable to newly-introduced animals (Buck and Spotte 1986).

Our experience suggests that it is appropriate to sample the environment from which animals for captivity are taken and to monitor the animals frequently over extended periods for the presence of potentially pathogenic organisms. These data are necessary for proper husbandry techniques for marine mammals in captivity.

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Biomass, Size Structure, and Composition of Phototrophic and Heterotrophic Nanoflagellate Communities in Lakes Huron and Michigan[1]

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Carrick, H. J., and G. L. Fahnenstiel. 1989. Biomass, size structure, and composition of phototrophic and heterotrophic nanoflagellate communities in lakes Huron and Michigan. Can. J. Fish. Aquat. Sci. 1922–1928.

The abundance and biomass of surface (5 m) and deep (20–45 m) nanoflagellate communities in Lakes Huron and Michigan were determined during 1987. Abundances (10^2–10^3 cells·mL^{-1}) were comparable between lakes and similar to those reported from other oligotrophic environments. Community composition was skewed towards the small end of the size spectrum due to the prevalence of small chrysomonads. In general, heterotrophic flagellates (Hnano) were more abundant that phototrophic flagellates (Pnano), while standing stocks of Pnano carbon (average 24.7 μg C·L^{-1}) were greater than Hnano carbon (9.6 μg C·L^{-1}) on nearly all sample dates. The abundance of nanoflagellates in both Lakes Huron and Michigan peaked in July, perhaps indicating increased growth at higher temperatures and/or a response to higher abundance of prey. Nanoflagellate communities in deep waters during thermal stratification were more abundant (50–70% higher carbon) than surface communities and were dominated by Pnano. High carbon standing stocks of deep communities did not correspond with high prey abundances. Thus, deep communities seem to be influenced by factors (e.g., light and nutrients) that maintain deep phytoplankton communities in the upper Great Lakes. While Hnano are quantitatively important in Lakes Huron and Michigan, representing nearly 20% of phytoplanton biomass, their trophic role is largely unknown.

L'abondance et la biomasse de la communauté de nanoflagellés de surface (5 m) et d'eaux profondes (20–45 m) des lacs Huron et Michigan ont été déterminées au cours de 1987. Les abondances (10^2 à 10^3 cellules·mL^{-1}) étaient comparables pour les deux lacs et semblables aux abondances observées dans d'autres milieux oligotrophes. La composition de la communauté était biaisée vers les petites tailles à cause de la présence majoritaire de petites chrysophycées. En général, les flagellés hétérotrophes (Hnano) étaient plus nombreux que les flagellés phototrophes (Pnano) et les stocks de carbone dérivés des Pnano (en moyenne 24,7 μg C·L^{-1}) étaient supérieurs à ceux dérivés des Hnano (9,6 μg C·L^{-1}), presque toutes les fois où des échantillonnages ont été réalisés. L'abondance de nanoflagellés dans les lacs Huron et Michigan était à son maximum en juillet, à cause peut-être des températures plus élevées et/ou d'une plus grande abondance de proies. Les communautés de nanoflagellés étaient plus abondantes dans les eaux profondes (50 à 70 % de plus de carbone) qu'en surface et étaient constituées surtout par des Pnano. Les stocks élevés de carbone des communautés des eaux profondes ne correspondaient pas à une abondance élevée de proies. Les communautés des eaux profondes semblent donc être touchées par d'autres facteurs (par exemple lumière et éléments nutritifs) qui expliquent la présence de communautés de phytoplancton en profondeur dans le secteur supérieur des Grands Lacs. Bien que les Hnano soient nombreux dans les lacs Huron et Michigan, représentant près de 20 % de la biomasse de phytoplancton, le rôle trophique qu'ils jouent est très peu connu.

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Picoplankton (organisms less than 2.0 μm in length) are important contributors to total productivity of aquatic ecosystems and are a significant and common feature of many marine systems (e.g., Li et al. 1983; Fuhrman et al. 1985; Cole et al. 1988) and oligotrophic lakes (Fahnenstiel 1980; Cole et al. 1984; Riemann and Sondergaard 1984; Stockner and Antia 1986). This recently discovered source of productivity has caused a reevaluation of existing concepts of trophic structure within aquatic habitats (e.g., Azam et al. 1983). In the Upper Great Lakes as well as in marine systems, much of the primary production (annual average > 50%) can be attributed to organisms, primarily cyanobacteria, which pass a through 3-μm screen (Fahnenstiel et al. 1986; Fahnenstiel, unpubl. data). In addition, heterotrophic picoplankton, primarily bacteria, are responsible for a large portion of carbon flux (Scavia et al. 1986b; Scavia and Laird 1987) and nutrient recycling (Gardner et al. 1986, 1987).

However, the importance of heterotrophic nanoplankton (Hnano, flagellates and ciliates 2–20 μm in diameter) in aquatic ecosystems was not realized until recently (Sherr and Sherr 1984). Determinations of Hnano abundance, carbon standing

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stock, and seasonal dynamics (e.g., Sorokin 1977; Beers et al. 1982; Sherr et al. 1984; Davis et al. 1985) indicate that these organisms are abundant components of marine plankton and have high metabolic rates (Fenchel 1982a; Sieburth 1984). Furthermore, marine Hnano significantly graze on picoplankton (Fenchel 1982b; for review see McManus and Fuhrman 1988b), control the flux of carbon within the microbial food web (Newell and Linley 1984; Pomeroy 1980), recycle nutrients (Goldman et al. 1985; Anderson et al. 1986), decompose particulate organics (Sherr et al. 1982), and appear to represent the dominant link between the microbial food web and higher trophic levels (Pace et al. 1984; Porter et al. 1985; Sherr et al. 1986, 1987).

Conversely, there are few measurements of heterotrophic nanoplankton abundance and distribution in freshwaters (Pick and Caron 1987). Although much information exists concerning the relative importance and abundance of phototrophic nanoplankton in the Laurentian Great Lakes (Vollenweider et al. 1974; Munawar and Munawar 1975, 1981, 1982; Munawar et al. 1978; Fahnenstiel and Scavia 1987a), Hnano biomass has only recently been estimated in Lake Ontario (Pick and Caron 1987), where they represent nearly 20% of total phytoplankton biomass (compared with phytoplankton biomass in Gray 1987). Here we report the abundance, size structure, and composition of phototrophic (Pnano) and heterotrophic (Hnano) nanoflagellate communities from an offshore station in both Lakes Huron and Michigan in 1987. We have restricted our analysis to nanoflagellates, because they are the dominant component of nanoplankton in the Upper Great Lakes (Munawar and Munawar 1981) and are potential grazers on picoplankton (Caron et al. 1985; Fahnenstiel et al. 1986; Scavia et al. 1986b).

Materials and Methods

Sampling was conducted in 1987 at single offshore stations in Lake Huron (43° 56′ N, 82° 21′ W; max. depth = 70 m) and Lake Michigan (43° 1′ 11″ N, 86° 36′ 48″ W; max. depth = 100 m) and on one occasion in 1986 (8 December) from the Lake Michigan station. The biological, chemical, and physical conditions at both stations are representative of offshore conditions (Makarewicz 1985, 1987), with ambient concentrations of total phosphorus being 5 and 8 $\mu g \cdot L^{-1}$ in Lakes Huron and Michigan, respectively (Schelske et al. 1986). In both lakes, phytoplankton standing stocks are low, typically less than 50 $\mu g\ C \cdot L^{-1}$, and chlorophyll-*a* concentrations range from 0.3 to 3.0 $mg \cdot m^{-3}$ (Fahnenstiel et al. 1989). Also, bacterial abundances range from 0.5×10^6 to 1.0×10^6 cells$\cdot mL^{-1}$, while autotrophic picoplankton span from 1.0×10^4 to greater than 10×10^4 (Scavia et al. 1986b; G. L. Fahnenstiel, NOAA, 1986–89 unpubl. data).

Monthly samples (December 1986–November 1987) from both lakes were collected from the surface when the water column was isothermal, and from both the surface (5 m) and deep (20–45 m) regions during thermal stratification, using opaque Niskin (5 L) sampling bottles. Subsamples were transferred into 250-mL amber bottles and preserved with glutaraldehyde (0.20 M final conc.) buffered with sodium cacodylate (0.1 M final conc.). Water column temperature profiles were measured with an electronic bathythermograph, and chlorophyll-*a* concentrations were determined fluorometrically (Strickland and Parsons 1972).

Samples were stored cold (5°C) prior to preparation for epifluorescence microscopy, which was performed within 24 h after sampling following the method of Caron (1983). Once prepared, slides were immediately stored at −20°C, and were counted within one month to minimize the fading of autofluorescence (Caron 1983). Flagellate biomass and composition were estimated by enumerating 400–500 individuals from each prepared slide (< 5% counting error assuming Poisson statistics), using a Leitz Laborlux Microscope (mag = 1250×) equipped for autofluorescence (450–490 nm excitation and > 515 nm emission), and primulin analysis (320–380 nm excitation and > 420 nm emission). The length and breadth of individual organisms counted were determined. In addition, we observed trophic status (pigmented = phototrophic and non-pigmented = heterotrophic), the arrangement of external structures (i.e., flagella), and body shape. Cell volumes were calculated for each taxon assuming approximate geometric configurations; volume estimates for Pnano were subsequently converted to carbon based on Strathman (1966) conversion factors while Hnano cell volumes were converted to carbon using the conversion factor (cell volume × 0.15 g $C \cdot mL^{-1}$) of Laws et al. (1984).

Results

Seasonal Abundance and Biomass

The abundance (cells$\cdot mL^{-1}$) of surface Hnano communities exhibited strong seasonality in both Lakes Huron and Michigan, while Pnano numbers showed little seasonal variation (Fig. 1A and 1C). In Lake Huron, Hnano abundances were greater than Pnano on all dates sampled, varying from 0.7×10^3 to 1.5×10^3 cells$\cdot mL^{-1}$ in the spring to over 5.0×10^3 cells$\cdot mL^{-1}$ during the summer. Pnano abundance ranged from 0.4×10^3 to 1.2×10^3 cells$\cdot mL^{-1}$ during isothermal periods (winter through spring) to more than 1.2×10^3–1.6×10^3 cells$\cdot mL^{-1}$ following thermal stratification. In Lake Michigan, the abundance of Hnano was also greater than that of Pnano for almost all dates sampled, as spring standing stocks were low (0.6×10^3–1.2×10^3 cells$\cdot mL^{-1}$), and increased more than two-fold during the summer (2.4×10^3–5.0×10^3 cells$\cdot mL^{-1}$). Pnano winter–spring standing stocks were 0.3×10^3–0.8×10^3 cells$\cdot mL^{-1}$ and only increased to between 1.4×10^3–1.9×10^3 cells$\cdot mL^{-1}$ during stratification.

Although Hnano had higher cell abundances than Pnano, Hnano carbon was lower than Pnano carbon on nearly all sampling dates and comprised 25.1 and 31.0% of total nanoflagellate carbon in Lakes Huron and Michigan, respectively (Fig. 1B and 1D). Nanoflagellate carbon in Lake Huron demonstrated little seasonality, with values for Hnano ranging from 2–8 $\mu g\ C \cdot L^{-1}$ to 9–13 $\mu g\ C \cdot L^{-1}$ in the spring and summer, respectively, while Pnano carbon varied from 13–16 $\mu g\ C \cdot L^{-1}$ in the spring to 23–38 $\mu g\ C \cdot L^{-1}$ following stratification. Nanoflagellate biomass showed more pronounced seasonality in Lake Michigan. Hnano carbon ranged from 2–6 $\mu g\ C \cdot L^{-1}$ in the spring to 8–24 $\mu g\ C \cdot L^{-1}$ during the summer, while spring Pnano carbon increased from 7–19 $\mu g\ C \cdot L^{-1}$ to 10–56 $\mu g\ C \cdot L^{-1}$ during stratification.

The abundance (cells$\cdot mL^{-1}$) of deep communities of Pnano and Hnano in both lakes increased between two- and three-fold during mid-stratification (July) compared with standing stocks estimated during early- (June) and late- (Oct.–Nov.) stratification (Table 1). Although the abundance of Lake Huron Pnano and Hnano were comparable, Pnano carbon was three- to four-fold higher than Hnano carbon. Similarly, Lake

TABLE 1. Abundance (cells·mL^{-1}) and carbon content (μg C·L^{-1}) of autotrophic and heterotrophic nanoflagellate communities from the deep (20–45 mm) region in Lakes Huron and Michigan during the stratified period. The ratio of Pnano:Hnano carbon is also presented.

		Pnano		Hnano		
Lake	Date	Abundance	Carbon	Abundance	Carbon	Carbon Ratio
Huron	10 June	1741	39.30	2515	18.83	2.09
	29 July	6090	86.50	5475	22.72	3.81
	15 Oct.	746	31.42	1466	7.64	4.11
Michigan	22 June	973	24.39	1682	8.38	2.91
	21 July	2972	33.38	4101	23.02	1.45
	20 Aug.	979	10.12	5197	23.71	0.42
	18 Sept.	221	5.92	947	3.67	1.61
	2 Nov.	443	12.80	1666	7.07	1.81

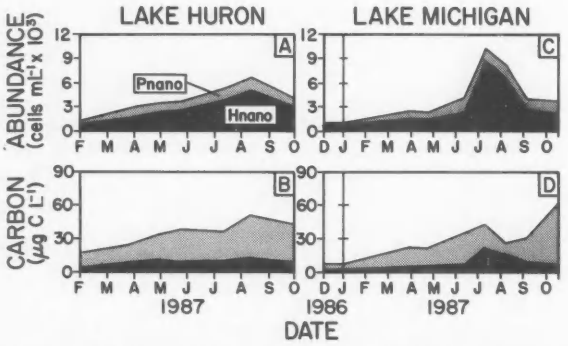

FIG. 1. Monthly estimates of surface (5 m) nanoflagellate abundance (A and C) and carbon content (B and D) for phototrophic (Pnano) and heterotrophic (Hnano) communities in Lake Huron (Feb.–Oct. 1987) and Lake Michigan (Dec. 1986–Nov. 1987).

Michigan Pnano carbon was 1.5 to nearly three times higher than Hnano carbon, with the exception of one date when the Pnano:Hnano carbon ratio was less than one (20 August), even though the abundance of Hnano was higher than that of Pnano on all dates sampled.

Size Structure

Seasonal frequency distributions of nanoflagellate size (ESD) for all sampling dates were skewed towards the small size classes (2–4 μm) with no major shifts between size classes (Fig. 2A–2H). The average ESD ± one standard deviation for surface communities was 3.06 ± 0.26 μm for Lake Huron and 3.26 ± 0.39 μm for Lake Michigan. The size structure of deep nanoflagellate communities from both lakes (Fig. 3A–3F) was also skewed towards the small end of the ESD size spectrum and showed a small degree of seasonal variation among the sampling dates (Lake Huron 3.34 ± 0.06; Lake Michigan 3.16 ± 0.20). Moreover, Hnano in general were smaller than Pnano in both surface and deep communities; a greater percentage of Hnano had ESDs which were in the 2–7 μm range, while most Pnano had ESDs greater than 7 μm (Fig. 4).

Community Composition

In both lakes, surface Pnano biomass was dominated by cryptomonads (i.e., *Rhodomonas minuta* Skuja, *Rhodomonas lens* Pasch. Ruttner, *Cryptomonas erosa* Ehr., and *Cryptomonas* sp.) throughout most of the year, with chrysomonads

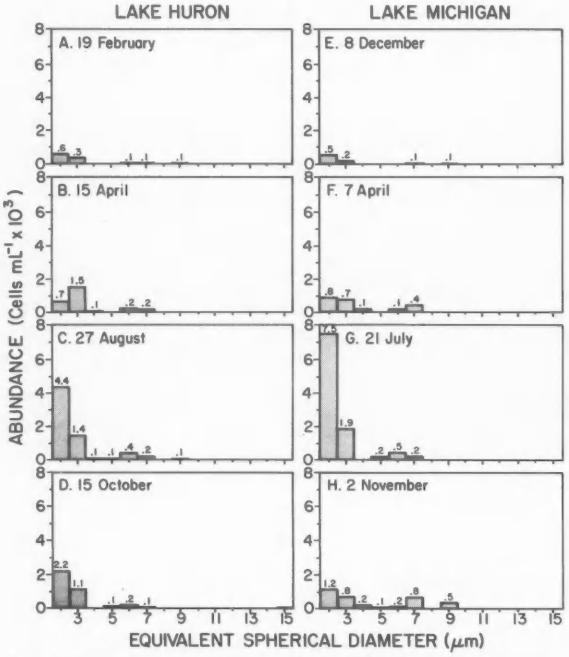

FIG. 2. Typical size (equivalent spherical diameter, μm) frequency distributions for surface (5 m) nanoflagellate communities in Lakes Huron (A–D) and Michigan (E–H).

and other small flagellates (*Dinobryon divergens* Imhof., *Chrysochomulina parva* Lackey, *Ochromonas* spp., and unidentified flagellates) becoming more important following stratification. In Lake Huron, the biomass of chrysomonads and small flagellates gradually increased during the year, whereas a chrysomonad peak in Lake Michigan occurred between 22 June and 21 July, followed by a pronounced increase in cryptomonad biomass on 2 November.

Hnano community composition was also similar between lakes and was composed primarily of colorless cryptomonads and chrysomonads. In Lake Huron, Hnano composition showed little seasonality. Hnano biomass contained on average 35.0% cryptomonads (*Katablepharis ovalis* Skuja, *Cryptaulax conoidea* Skuja, and *Chroomonas* sp.) and 36.2% chrysomonads (e.g., *Ochromonas* spp., *Chromulina* sp., *Monas* sp., and small unidentified flagellates), while other zooflagellates (mainly

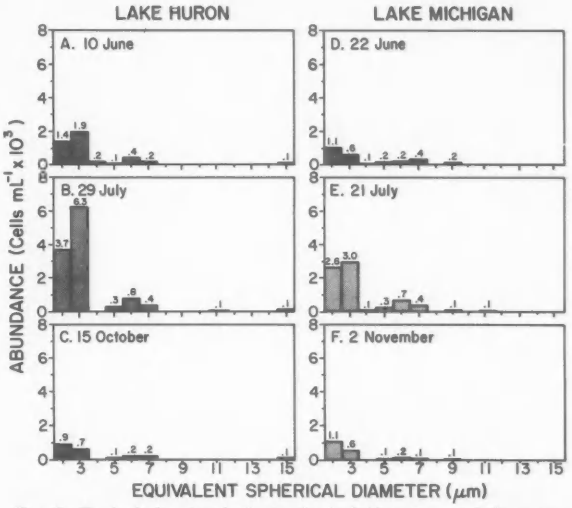

FIG. 3. Typical size (equivalent spherical diameter, μm) frequency distributions for deep (20–45 m) nanoflagellate communities in Lakes Huron (A–C) and Michigan (D–F).

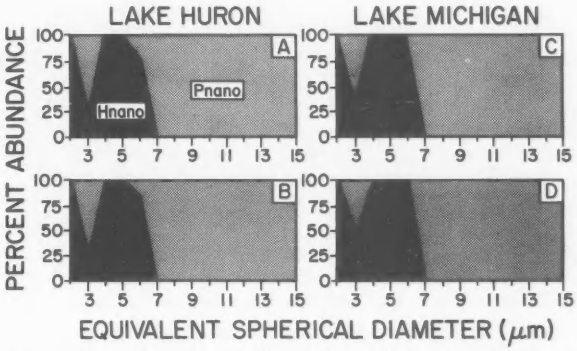

FIG. 4. Percent abundance (cells·mL^{-1}) as a function of size (based upon equivalent spherical diameter, μm) for surface (A and B) and deep (C and D) heterotrophic (Hnano) and phototrophic (Pnano) nanoflagellate communities in Lakes Huron and Michigan. Percent abundances were averaged over all sampling dates and the standard deviations for estimates in any one size class were less than 24%.

choanoflagellates such as *Monosiga* sp. and *Diploeca* sp.) were subdominant. Similarly in Lake Michigan, cryptomonad populations constituted most of the Hnano biomass (37.2%) and varied little with season, while chrysomonads (35.2%) and other zooflagellates (17.7%) increased after 21 July (mid-stratification) and were responsible for much of the seasonality in Hnano biomass.

The composition of deep-water Pnano communities was similar to that in the surface waters with some exception. Cryptomonads dominated these assemblages; however, increases in Pnano carbon during mid-stratification in both lakes were primarily attributed to the increased biomass of chrysomonads (e.g., *Chrysochromulina parva* Lackey) and dinoflagellates (*Gymnodinium varians* Maskell and *Gymnodinium* sp.), which comprised 46.8 and 6.0%, respectively, of Pnano carbon. Moreover, the community structure of deep Hnano was also similar in both lakes, where cryptomonads, chrysomonads, and other zooflagellates were co-dominant during early- and late-stratification periods, although communities shifted towards cryptomonad and chrysomonad dominance during mid-stratification.

Discussion

In general, the abundance (overall average ± SD, 4.44 ± 2.28 × 10^3 cells·mL^{-1}) and biomass (34.2 ± 14.2 μg C·L^{-1}) of surface nanoflagellate communities were comparable between Lakes Huron and Michigan, and they were also similar to those reported from other oligotrophic environments. These values agree with nanoflagellate standing stocks in Georgia coastal waters (Sherr et al. 1984), open oceans (Davis and Sieburth 1982), the North Atlantic (Davis et al. 1985), and the North Sea (Fenchel 1982b).

Hnano were more numerous than Pnano in both lakes in nearly all the samples analyzed (average Pnano:Hnano Lake Huron = 0.53, Lake Michigan = 0.59). Other workers have also observed higher or similar abundances (cells·mL^{-1}) of Hnano compared to Pnano in marine systems, with Pnano:Hnano ratios being equal to or less than 1.0 (Caron 1983; Davis and Sieburth 1982; Davis et al. 1985; Sherr et al. 1984). This was not observed in the only other study of a Laurentian Great Lake, in which Pick and Caron (1987) found higher concentrations of Pnano compared with Hnano in Lake Ontario. While Pick and Caron's biomass estimates for Hnano (taken from their Fig. 4, midlake station 403) were comparable to ours (0.083 and 0.063 g·m^{-3}, respectively), their biomass estimates for Pnano were two-fold higher than those presented here (0.33 and 0.15 g·m^{-3}, respectively). The prevalence of non-flagellated Pnano (coccoid green algae and diatoms) seems to account for the greater abundance of Pnano measured in Lake Ontario (Pick and Caron 1987). These forms were not as abundant in our samples and hence were not included in our estimates. Such differences in nanoplankton community composition may reflect the more eutrophic nature of Lake Ontario relative to Lakes Huron and Michigan (Schelske et al. 1986).

Conversely, we found that Pnano carbon in the surface waters of both Lakes Huron and Michigan were consistently greater than Hnano carbon (average carbon Pnano:Hnano Lake Huron = 3.31, Lake Michigan = 2.61). This result is not surprising given that Pnano, despite their lower abundance, are larger than Hnano (see Fig. 4). The skewed size structure of nanoflagellates in these lakes can also be attributed to the abundance of colorless chrysomonads, 2–4 μm in equivalent spherical diameter, which represent nearly 70% of nanoflagellate abundance. This feature has been commonly observed in oligotrophic marine and freshwater systems (Sherr and Sherr 1983; Rassoulzadegan and Sheldon 1986; Anderson 1987). The majority of these taxa probably belong to the genera *Ochromonas* and *Chromulina* based upon body shape and flagellation. However, the identity of these flagellates is difficult to ascertain and requires confirmation with transmission electron microscopy (Estep et al. 1986).

Seasonal Variation in Nanoflagellate Biomass

The surface abundance of nanoflagellates peaked during mid-stratification in Lake Michigan and to a lesser extent in Lake Huron, primarily because of a dramatic increase in the abundance of small chrysomonads. This is similar to seasonal variation in nanoplankton communities previously observed in

other temperate aquatic systems (Fenchel 1986) and in the Great Lakes (Munawar et al. 1978; Pick and Caron 1987). Positive correlations between nanoflagellate abundance and temperature for both Lakes Huron and Michigan ($r=0.90$, $P=0.006$; $r=0.84$, $P=0.009$, respectively) may reflect higher nanoflagellate growth rates at higher temperature, as some marine nanoflagellates demonstrate increased growth (Caron et al. 1986) and metabolism (Sherr et al. 1988) at higher temperatures.

The seasonal increase in Lake Michigan nanoflagellate abundance observed here coincides with increases in populations of potential prey. The abundance, growth rates, and carbon production of Lake Michigan bacteria (Scavia and Laird 1987) and autotrophic picoplankton (G. L. Fahnenstiel, NOAA, 1987 unpubl. data) are also maximal during mid-stratification. Moreover, Hnano abundance in Lake Michigan was strongly correlated ($r=0.92$, $P=0.001$) with bacterial abundance (H. J. Carrick, NOAA, 1987 unpubl. data), perhaps suggesting a predator-prey relationship, in that small flagellates have been implicated as the major consumers of bacteria in Lake Michigan (Scavia et al. 1986b; Scavia and Laird 1987). This relationship was not observed in Lake Huron, probably due to the relatively small degree of seasonal variation in concentrations in Hnano (this study) and bacteria (H. J. Carrick, NOAA, 1987 unpubl. data).

The subtle seasonal variation in Lake Huron nanoflagellate abundance is intermediate to that occurring in Lake Superior, which shows little to no seasonal variation (Munawar et al. 1978; Fahnenstiel and Glime 1983; Fahnenstiel et al. 1986), and in Lake Michigan, which demonstrates more pronounced flagellate seasonality (Fahnenstiel and Scavia 1987a; this study). This trend is reflected in the variation in phytoplankton standing stocks (measured as chlorophyll-*a*) in the three lakes; values for offshore Lake Huron stocks (1.4–2.2 $mg \cdot m^{-3}$) lie between the values determined for Lake Superior (ca. 1.0 $mg \cdot m^{-3}$ little variation) and Lake Michigan (0.3–5.3 $mg \cdot m^{-3}$) (Vollenweider et al. 1974). Also, seasonal variation in parameters like total phosphorus concentrations and silica utilization in Lake Huron are intermediate to those measured in Lakes Superior and Michigan (Schelske et al. 1986). This is not surprising given that Lake Huron receives a mix of water from both lakes Michigan and Superior (Schelske 1985) and appears to have an intermediate trophic status among the three upper Great Lakes (Schelske et al. 1983, 1986).

Vertical Variation: Comparison of Surface and Deep Communities

Vertical heterogeneity in nanoflagellate communities has been observed in some marine habitats (Townsend and Cammen 1985; Kimor et al. 1987; Eppley et al. 1988), whereas little vertical structure was observed in Lake Ontario (Pick and Caron 1987). Based upon our results, the average biomass of deep nanoflagellate communities were 73 and 56% higher than that of surface communities in Lakes Huron and Michigan, respectively (Table 4), and were dominated by phototrophic flagellates (average carbon Pnano:Hnano = 2.37, SD = 1.26). The development of this subsurface nanoflagellate community was greatest during mid-stratification, while decreases in the deep:surface carbon ratio after July were related to increasing mixing depth and the eventual mixing of the subsurface community (Table 2). This progression was also evidenced by the diminishing ratio of deep:surface chlorophyll-a estimates (values < 1.5), indicating a decline of the deep-water phytoplankton community in each lake (Fahnenstiel and Scavia 1987b). The vertical distribution of nanoflagellates observed here is similar to that determined for phytoplankton in Lake Michigan (Fahnenstiel and Scavia 1987b) and Lake Superior (Fahnenstiel and Glime 1983), and for autotrophic picoplankton in lakes Huron and Michigan (G. L. Fahnenstiel, NOAA, 1987 unpubl. data). However, there does not appear to be a concomitant increase in bacteria at this depth in the water column (Scavia and Laird 1987) for Lake Michigan. Thus, it seems more likely that deep nanoflagellate communities are influenced more by factors such as light and nutrients, which regulate subsurface phytoplankton in Lake Michigan (Fahnenstiel et al. 1984). The lower light penetration in Lake Ontario (1% light at 11.5 m, Cuhel and Lean 1987) compared with that in Lakes Huron and Michigan (20–30 m, G. L. Fahnenstiel, NOAA, 1987 unpubl. data; Scavia et al. 1986a, respectively) might account for the lack of vertical heterogeneity in nanoflagellates in Lake Ontario compared with the results presented here.

Trophic Role of Nanoflagellates in the Upper Great Lakes

Hnano are quantitatively important in both Lakes Huron and Michigan, representing nearly 20% of phytoplankton carbon (compared with G. L.Fahnenstiel, NOAA, 1987 unpubl. data), which is similar to that observed for Lake Ontario communities (Pick and Caron 1987). However, the role of nanoflagellates in the upper Great Lakes food web is unclear. Given the present information, we can estimate Hnano clearance rates, assuming a constant clearance of 1×10^5 cell volumes·flagellate^{-1}·h^{-1} (Fenchel 1982a). Thus, surface Hnano (average biovolume 23.6 μm^3) in both lakes (average abundance 2.9×10^3 cells·mL^{-1}) clear on average 2.4 nL·flagellate^{-1}·h^{-1}, which is similar to rates determined elsewhere for species of equivalent size (Fenchel 1982a; Sherr et al. 1983). This clearance rate accounts for

TABLE 2. Comparisons of total nanoflagellate carbon ($\mu g\ C \cdot L^{-1}$) and chlorophyll-*a* concentration ($mg \cdot m^{-3}$) between surface (5 m) and deep (20–45 m) communities in Lakes Huron and Michigan for several dates during the stratified period.

Lake	Date	Surface Carbon	Deep Carbon	Carbon Deep:Surface	Chlorophyll Deep:Surface
Huron	10 June	38.85	53.13	1.37	1.93
	29 July	37.05	109.20	2.95	11.03
	15 Oct.	44.51	39.06	0.88	0.69
Michigan	22 June	30.68	32.77	1.07	4.19
	21 July	21.20	56.40	2.65	2.36
	20 Aug.	10.28	33.83	3.29	1.47
	18 Sept.	22.88	9.59	0.42	—
	2 Nov.	56.39	19.87	0.35	0.34

20% of bacterial loss rates previously determined in Lake Michigan which has been attributed to micrograzers (Scavia and Laird 1987; Scavia and Fahnenstiel 1988). This estimate is low compared to most other studies (e.g., Fenchel 1982a; Landry et al. 1984), although some investigations have observed similar rates of bacterial grazing by Hnano (McManus and Fuhrman 1988a). However, it should be noted that small naked flagellates can undergo nearly 50% size reduction following preservation with glutaraldehyde, as well as other standard fixatives (Borsheim and Bratbak 1987; Bloem et al. 1988). Hnano shrinkage was not investigated here nor was 50% size reduction accounted in our biomass estimates, and subsequent clearance rates. Thus, doubling Hnano clearance to account for size reduction following preservation, Hnano can account for nearly 40% of bacterial losses. Moreover, if we assume that Pnano are active bacterial grazers in Lake Michigan as reported elsewhere (Bird and Kalff 1986, 1987; Porter 1988) and include them in our calculation (Pnano clearance rate 12.6 $nL \cdot flagellate^{-1} \cdot h^{-1}$), approximately 50% of bacterial losses in Lake Michigan can be attributed to nanoflagellate grazing. This suggests that other pelagic microzooplankton (i.e., ciliates and rotifers) in addition to heterotrophic nanoflagellates, are active in controlling bacterial populations in Lake Michigan. For instance, ciliated protozoa can be significant bacterivores in both marine (Sherr and Sherr 1987; Sherr et al. 1987) and freshwater (Simek et al. 1988) systems. In addition, Hnano in the Great Lakes might utilize other nutritional resources, as they have been shown to be important grazers of autotrophic picoplankton (Campbell and Carpenter 1986) and other algae (Goldman and Caron 1985; Suttle et al. 1986), and are equipped to use dissolved organic matter as an additional nutritional source (Sherr 1988). Although the contribution of nanoflagellate biomass to upper Great Lakes plankton is now evident, their role in the Great Lakes food web is not presently understood and merits further study.

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Changes in Conductivity, Alkalinity, Calcium, and pH During a 50-Year Period in Selected Northern Wisconsin Lakes

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Between 1925 and 1941, E. A. Birge, C. Juday, and co-workers in Wisconsin made extensive water quality measurements on over 600 northern Wisconsin lakes, providing a benchmark against which comparisons can be made if the modern equivalent measurements can be determined. Of these, 149 lakes were sampled from 1979 to 1983. Data on conductivity, alkalinity, calcium, and hydrogen ion (pH) are available for these lakes from 1925 to 1931. The historical methyl orange alkalinity data were adjusted to be comparable to the recent Gran alkalinity data by subtracting 57 μmol/L from the historical measurements. The colorimetric historical pH measurements were adjusted to be comparable to recent potentiometric measurements by a regression model. No adjustments were made to the historical calcium or conductivity measurements. Comparisons of adjusted historical data with recent data show a significant increase in the means of all four variables. The largest increases in all four variables show an association with increases in land use development on the lake perimeters. Statistically significant changes in alkalinity are observed, with 28 lakes increasing and 11 decreasing, compared with 110 lakes showing no change. Possible explanations for chemical changes in those lakes that have experienced little change in land use are discussed. For the lakes in northern Wisconsin which may have been affected by airborne pollutants, it is likely that these effects had their beginning before initiation of the historical lake survey.

Entre 1925 et 1941, E. A. Birge, C. Juday et leurs collègues ont réalisé un nombre considérable de mesures de la qualité de l'eau dans plus de 600 lacs du nord du Wisconsin; ces mesures, véritables points de référence, permettent d'établir des comparaisons là où les mesures actuelles équivalentes peuvent être déterminées. Ainsi, 149 des lacs déjà échantillonnés, l'ont été de nouveau entre 1979 et 1983. Il existe des données pour ces lacs sur la conductivité, l'alcalinité et la teneur en calcium et en ions hydrogènes (pH) pour la période de 1925-1931. On a soustrait 57 μmol/L des données d'alcalinité de cette époque (méthylorange) pour permettre de les comparer aux données récentes (méthode de Gran). Un modèle de régression a servi à rendre comparable les mesures colorimétriques précédentes du pH aux mesures potentiométriques récentes. Les mesures de conductivité ou de teneur en calcium les plus anciennes n'ont pas fait l'objet de corrections. Les comparaisons de ces données corrigées et des données récentes indiquent une augmentation significative de la moyenne pour les quatre variables. Les plus importantes augmentations, chez les quatre variables, sont liées à un accroissement de l'utilisation des terres en bordure des lacs. Des variations statistiquement significatives de l'alcalinité ont été observées : l'alcalinité augmente dans 28 lacs, diminue dans 11 et reste semblable dans 110. On présente les raisons possibles expliquant les variations chimiques observées dans les lacs autour desquels l'utilisation des terres a très peu changé. Dans le cas des lacs du nord du Wisconsin qui auraient pu être touchés par les polluants atmosphériques, il est probable que ces effets aient commencé à se faire sentir avant le premier échantillonnage.

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The acidification of lakes in industrialized countries of Europe and North America caused by atmospheric deposition has been well documented (Martin 1987). Within the Upper Midwestern portion of the United States, several investigators have identified lakes which also appear, in part, to have been acidified by atmospheric deposition (Schnoor et

al. 1986; Nichols and McRoberts 1986; Eilers et al. 1988). Although the sulfate deposition in the eastern United States has been decreasing in the last decade, large portions of the United States continue to receive deposition acidified by anthropogenic sources (National Research Council 1986; Glass et al. 1986). Northern Wisconsin receives an annual precipitation of 80 cm, with an average pH of 4.7 (1979–82) and an estimated SO_4^{2-} wet deposition rate of 17 kg/ha (Glass and Loucks 1986). These values in Wisconsin approach the pH of precipitation observed to cause lake acidification in Norway (Wright and Henriksen 1978).

From 1925 to 1941, Birge, Juday, and co-workers amassed water quality data on over 600 northern Wisconsin lakes (Frey 1966). The common water chemistry measures (e.g., pH, conductivity, and alkalinity) available for many of these lakes provide a benchmark against which direct comparisons can be made with recent lake water quality data if the modern equivalents of these historical measurements can be determined. Thus, in proposing to examine whether acidification or alkalinization of lakes has taken place in northern Wisconsin between the historical and recent periods, several questions must be explored. The first of these is methodological. What considerations are necessary before presenting historical data in a form comparable to equivalent modern data within a specifiable confidence limit? The second question applies to the gross patterns of water quality changes that may be found over the 50-yr period. What water quality changes have been observed in what lakes, and what methods are appropriate for summarizing the observations? Finally, how has the broad and sometimes opposing mix of anthropogenic alterations (logging, regrowth, cultural eutrophication, and acidic deposition) combined with a variety of mediating processes within the watershed (hydrologic, geochemical, and biotic) to produce the responses observed? Fortunately, the rich 1930's data base for northern Wisconsin and a substantial recent record allow some of these questions to be addressed.

The objective of this paper is to examine the first two of the issues raised above: methodological problems, and overall patterns of change. The paper also develops a general background for evaluation of the mechanisms by which the observed changes could have been induced.

Methods

A comparative analysis of chemical data on lakes over a period of 50 years requires, first, the assurance that the same lakes are being measured, and secondly, that the analytical methods are comparable.

Lake Identification

To satisfy the requirement that any comparisons of lake chemistry must be made with data from the same lake, we first confirmed lake identification. Since the historical lake survey was initiated in the 1920's, approximately one-third of the lakes sampled by Birge and Juday have undergone changes in name. In addition, the study area contains several thousand lakes and, as a consequence, there are numerous cases of duplication of lake names. Because of the changes and redundancy in names, other information was required to confirm the identity of a lake. For example, lake morphometry, proximity to landmarks, and legal descriptions were used to confirm lake identification including the notes, hand-drawn maps, and location descriptions of the original investigators, land use economic inventory maps of the period, 1925 county plat books, county surface water resources inventories, early USGS planimetric maps, aerial photographs, early county soil surveys, and the official record of lake and stream name changes. Conflicts in lake identification among these sources were sometimes resolved by an examination of the water chemistry data where, for example, a softwater lake could easily be distinguished from a hardwater lake of the same name. The process of confirming lake identification led to the exclusion of several lakes from this comparison, because identification could not be made with certainty. The lakes were probably sampled by Birge and Juday, but there was insufficient evidence for confirmation. The documentation for the lake identification task is described more fully in Johnson (1984).

Selection of Study Lakes

The lakes were initially selected for comparison based on the availability of both historical pH and alkalinity data for lakes sampled from 1925–1941 and recent data from summer and fall surveys in 1979 (Eilers et al. 1983a). However, we sought to increase the number of softwater lakes available for comparison by sampling an additional 59 lakes in the summer of 1980 for which historical data were available. The 1980 survey was also designed to address questions of temporal and spatial variability in the lakes and differences in analytical methodology. Those softwater lakes, defined as historical alkalinity < 200 μmol/L, that had multiple historical observations were favored over other lakes. The recent period of record was extended by including data for several lakes through the summer of 1983. Of the 161 lakes with both recent and historical pH and alkalinity data, 10 lakes were excluded from the study based on suspected analytical errors in the historical data. Two lakes were excluded because of possible analytical errors in the recent data leaving 149 lakes available for comparison. A listing of the study lakes and the associated historical and recent data summarized here are available, at a nominal charge, from the Depository of Unpublished Data, CISTI, National Research Council of Canada, Ottawa, Canada K1A 0S2.

Historical Methods

Juday, Birge, and coworkers, as part of the Wisconsin Geological and Natural History Survey (WGNHS), sampled lakes in northern Wisconsin and compiled a database with about 2000 surface water observations on over 600 lakes (Juday and Birge 1930, 1933; Freeman et al. 1933; Meloche and Setterquist 1933; Juday et al. 1935, 1938). Although the data collection period spans 17 yr (1925–41), 72% of the surface observations were made during the first 6 yr of the survey. Over 90% of the samples were collected during the summer. The water samples were usually collected near the center of the lake with a brass Kemmerer sampler; one portion of the sample was used to fill a soda pop bottle with a tight spring-top cap for dissolved gas analysis while another portion was used to fill a BOD bottle and was covered with a rubber stopper. The samples were kept in the dark, unrefrigerated, and were transported to the Trout Lake Limnological Laboratory for analysis. Where analysis of cations or anions was desired, approximately 100 L was collected, transported to the laboratory in milk cans, and evaporated in porcelain dishes over gas stoves. Laboratory notebooks indicate that many of the samples were analyzed within 24 h for the most common parameters and were usually analyzed 1–3 h after

being returned to the laboratory (R. Pennak, University of Colorado pers. comm.).

Historical conductivity was measured with a portable Digby and Biggs Dionic Water Tester (Evershed 1910) and reported at 20°C. The meter they apparently used was a low-range model measuring from 0 to 200 μS/cm. The limit of error for this instrument "does not exceed 5%; usually it falls between 0 and 3%" (Juday and Birge 1933). A high-range (> 200 μS/cm) Dionic Water Tester was calibrated against standard KCl and NaCl solutions. No bias was observed with this instrument and we have no reason to suspect that the low-range meter did not measure accurately as reported (Emmling and Andren 1984).

Historical alkalinity was measured by adding methyl orange indicator and titrating a 100-mL sample with 1/44 *M* HCl to a "faint pink endpoint" (Juday et al. 1935). This general procedure was apparently followed throughout the lake survey project, except during 1932 when the samples were titrated to a deep, stable pink endpoint. The excess alkalinity from overtitrating during 1932 was corrected by subtracting the amount of acid required to neutralize the additional amount of indicator. Juday et al. (1935) refer to the alkalinity as a "field" measurement, but they apparently meant their field laboratory at Trout Lake as the site of measurement. This deduction is supported by the records of the laboratory technicians (University of Wisconsin Archives, Madison, WI).

The pH for most of the lake samples was measured at the Trout Lake Laboratory, although some measurements were made on the lake shore (Freeman et al. 1933). From 1925 to 1931, the pH was measured using a LaMotte colorimeter (Juday et al. 1935). During and following 1932, the investigators measured pH both colorimetrically and with a Youden portable meter (W. M. Welch, Co., Chicago). The accuracy of the Youden (quinhydrone) meter is discussed by Perley (1937) and is summarized by Welch (1948). Between 1933 and 1941, the investigators continued to measure pH colorimetrically and potentiometrically, but it is not always clear which method they used for a particular sample.

Historical calcium measurements were made using a modification of the McCrudden method as described in Meloche and Setterquist (1933). An analysis of 11 standard solutions containing 6 different concentrations of calcium yielded a standard error slightly less than 3.5 μmol/L with no significant bias reported (Meloche and Setterquist 1933). It is conceivable that the presence of organic matter could have resulted in a positive bias, but an examination of calcium versus conductivity by color (as a surrogate for organic matter) revealed no interaction. They also report results of an analysis of four dolomitic limestone samples from the U.S. Bureau of Standards with an average deviation within 0.15% of the standard, which would correspond to 3.5 μmol/L of the standard. An examination by Meloche and Setterquist of five paired samples of lake water showed a standard deviation of 9 μmol/L.

Birge, Juday, and co-workers also measured other ions (Cl^-, F^-, $NO_2^- + NO_3^-$, Mg^{2+}, Na^+, K^+), but in our opinion, serious deficiencies in the data prevented the use of this information for comparison. Sulfate was also measured in the historical period, but because of uncertainties in the holding times for some samples (records indicate that some of these samples were transported to Madison in milk containers before analysis) and because of possible interferences from the dissolved organics, we have not included these results for individual lakes. Nevertheless, a comparison of barium sulfate precipitation methodology similar to that used by Juday et al. (1938) with ion chromatography methodology showed no major differences (R. Cook, Oak Ridge National Laboratory pers. comm.). Consequently, we will present overall results comparing the unadjusted historical sulfate values with the recent data.

Recent Methods

The recent lake data that were compared with the WGNHS data were also surface samples collected in the summer (defined as June 30 to September 11 in both the historical and recent data). Unlike the historical samples, the recent samples were collected in 1-L Nalgene bottles (filled to prevent contact with air) and were kept on ice in the dark. Replicate samples were collected for analysis by two laboratories (1) Northern Lake Services, Inc. (NLS), Crandon, WI, and (2) the U.S.E.P.A. Environmental Research Laboratory (ERL-D), Duluth, MN.

Conductivity was measured in the ERL-D laboratory at or near 25°C with a YSI Model 32 meter. Temperature corrections were made using the method described by Sorensen and Glass (1987) and are discussed later. Alkalinity was measured by both ERL-D and NLS chemists. NLS staff measured alkalinity for duplicate samples using the standard EPA low-alkalinity method (U.S.E.P.A. 1979) and using two colorimetric indicator methods of bromcresol green-methyl red and methyl orange (APHA 1971). Because of the uncertainty in endpoint used by Birge and Juday, the recent methyl orange alkalinity was titrated to two endpoints at approximately pH 4.6 and 4.0 using 1/88 *M* H_2SO_4. Consistency in endpoints was maintained by titrating distilled water containing the indicator to the appropriate endpoint and using that color calibrated sample as a reference for the sample titrations. ERL-D alkalinity measurements are total inflection endpoint (TIP) values obtained using an automated titration with 0.010 *M* H_2SO_4, closed to the atmosphere, stirred and titrated to pH 3.8 using a Radiometer ABU 80 autoburette and Model M64 pH meter. All points with pH $\leq$ 4.5 are used in the Gran endpoint calculation of TIP alkalinity.

Field pH was measured with an Analytical Measurements (Model 707-B) meter with a gel-filled combination probe in 1979 and with an Orion 407A with a glass combination electrode in 1980–83. Field pH measurements served as a check on changes between field and laboratory values and were not used in the comparisons with the historical data. The pH meter used by NLS for laboratory pH was a Fisher Acumet (Model #230) with a gel-filled combination electrode (Fisher E5C). ERL-D used a Radiometer pH meter (Model #M64) with glass and reference electrodes. ERL-D pH measurements were chosen to represent the recent lake chemistry because they are internally consistent with their alkalinity and conductivity measurements. The colorimetric pH was measured at the NLS laboratory using a LaMotte pH color comparator with the following indicators and pH ranges: bromcresol green (4.0–5.4), bromcresol purple (5.2–6.6), bromthymol blue (7.0–7.4), and phenol red (6.8–8.4); the pH ranges and formulations (except bromcresol purple) have remained unchanged since the 1920's (R.H. Hutchinson, LaMotte Chemical, 1982, pers. comm.). Supplemental pH measurements were also performed on lake samples collected in 1982–83 using the following indicators: chlorphenol red (5.4–6.8), methyl red (4.4–5.8), and cresol red (7.2–8.6).

Although the colorimetric and TIP alkalinity measurements and colorimetric and potentiometric pH measurements were performed at different laboratories, comparison of conductivity and potentiometric pH values shows little difference. For example, ERL-D and NLS potentiometric pH values exhibited a

median difference of only 0.065 units and a Pearson correlation coefficient of 0.99.

Cation analysis was performed on acid-preserved aliquots at ERL-Corvallis using inductively coupled plasma/emission spectrophotometry with an Applied Research Laboratory ICP, Model QA 137, optimized for manganese with a sample uptake of 3 mL/min, argon consumption at 17 L/min. Subsequent analyses of the calcium measurements on the ICP indicate that these measurements may have a positive bias (relative to measurements on an atomic absorption spectrophotometer) of approximately 3 μmol/L. Anion analyses were performed at ERL-D using a Dionex ion chromatograph.

Adjustments for Methodological Differences

Conductivity — Conductivity was expressed by the WGNHS in μmhos/cm 20°C, whereas the recent data were recorded in μS/cm at 25°C. Temperature conversions for conductivity usually range between 2 and 3% per degree centigrade (i.e., 10–15% adjustment from 20° to 25°C) (Hem 1970; Lind 1979). Wagner (1971) found that a modified Walden conductance-viscosity relationship (Robinson and Stokes 1968) gave superior results over other temperature conversion methods for natural waters. The equation he used was $K \times \eta^A = B$ where K and η are the conductance and viscosity, respectively, at a given temperature and A and B are constants specific for each lake. This approach allowed us to convert accurately the recent conductivity measurements made at 25°C to conductivity at 20°C using a correction derived by computing the constant A based on the ionic composition of each lake (Sorensen and Glass 1987). The average absolute percent difference using this temperature conversion method compared with that of Juday and Birge (1933) is about 0.3% and is within the precision of their original measurements.

Alkalinity — Two common sources of differences between present and historical alkalinity measurements result from the methods of storing and titrating the sample. Kramer and Tessier (1982) discuss the importance of soft glass in contributing to the alkalinity of water samples left in storage before analysis. They report an average increase of about 13 $\mu mol \cdot L^{-1} \cdot d^{-1}$ over a 3-day period in samples stored in unrefrigerated, new glassware. We made no adjustments for possible alkalinity additions from the soft glass sample bottles used in the historical data set because the period between sample collection and analysis was generally less than one day, and the WGNHS sample bottles were probably well used.

There is clearly an adjustment required between the methyl orange alkalinity (MOA) measured in the historical data and the TIP titrations performed for the recent alkalinity, but little agreement has been achieved in determining what an appropriate conversion should be. Burns et al. (1981) subtracted 32 μmol/L (corresponding to an endpoint of pH 4.38) from the MOA values to compensate for colorimetric versus potentiometric alkalinity. Lewis (1982) assumed an endpoint of pH 4.4 (equal to an adjustment of 30 μmol/L) for the endpoint used by Pennak in the MOA titration. Zimmerman and Harvey (1979) concluded that no conversion could be applied to convert MOA to TIP alkalinity because the low alkalinity systems have significant buffering other than bicarbonate, and consequently, the titrations varied in an indeterminate manner. Kramer and Tessier (1982) found that the relationship between MOA and TIP alkalinity for a diluted Lake Ontario sample was consistent and could be adjusted by subtracting approximately 80 μeq/L from the MOA to equal the TIP titration results. Their analysis assumed that the pink endpoint (pH = 4.0) cited in Standard Methods (APHA 1971) was used. All the above investigators apparently assumed that the slope of the MOA-TIP relationship is one.

We investigated the relationship between MOA and TIP measurements by (1) measuring the MOA of standardized concentrations of $Ca(HCO_3)_2$, (2) measuring both MOA and TIP alkalinity for replicate lake samples, and (3) titrating lake samples potentiometrically with and without methyl orange indicator.

A comparison of standard solutions of calcium carbonate prepared using reagent grade material versus alkalinity measured with methyl orange indicator titrated to a pink endpoint (MOA_p) yields the following regression for alkalinity less than 200 μmol/L (all units in μmol/L):

$$(1)\quad MOA_p = 89\ (\pm 4) + 0.965\ (\pm\ 0.034)\ Ca(HCO_3)_2,$$
$$n = 24,\ r^2 = 0.99,\ P < 0.05,\ s = 12$$

The comparison of MOA_p and TIP alkalinity for multiple lake samples collected on 60 lakes in 1980 yields the following regression (all units in μmol/L):

$$(2)\quad MOA_p = 108\ (\pm\ 2) + 1.111\ (\pm\ 0.018)\ TIP,$$
$$n = 323,\ r^2 = 0.90,\ P < 0.05,\ s = 21$$

The intercept of 108 μmol/L is clearly much greater than one would expect based on the titration shown previously for standard solutions, and corresponds to a pH endpoint of 3.93 (assuming an endpoint of pH 5.00 for TIP). The endpoint derived from equation 2 contrasts sharply with analyses run on the same lake samples for an orange endpoint (MOA_o) (all units in μmol/L):

$$(3)\quad MOA_o = 37\ (\pm\ 1) + 1.042\ (\pm\ 0.015)\ TIP,$$
$$n = 328,\ r^2 = 0.94,\ P < 0.05,\ s = 16$$

The slope for MOA_o versus TIP is less than that for MOA_p versus TIP and is closer to the slope observed for the titrations with standard solutions (equation 1), indicating that the difference between the indicator method and TIP decreases slightly at higher alkalinity. However, the MOA_p bias for lake samples shows increasing departure from TIP at higher alkalinity. A possible cause for this increasing bias may be interference from color in these samples. A one-way ANOVA on the differences between MOA_p and TIP for low color ($\leq$ 20 PCU) and high color ($>$ 20 PCU) for the 1980 lake data shows that the high color group exhibits a 17 μmol/L additional bias ($F = 11.67$, $n = 320$). An analysis of residuals from a comparison of alkalinity and conductivity in the historical data shows a nonsignificant additional bias of 14 μmol/L for the high colored group. The standard deviation of the residuals increased among the color groups, doubling from lowest to highest colored lakes. By contrast, no color bias was observed for the MOA_o and TIP for the recent data.

An analysis of operator variability for MOA titrations showed significantly greater values (differences between MOA and TIP) for operator #2, who on both MOA_o ($F = 11.2$, $n = 327$) and MOA_p ($F = 9.2$, $n = 322$) measured 40 and 35 samples, respectively. Operator #3, who measured 150 samples, typically underestimated both MOA_o and MOA_p, relative to the other three operators. The standard deviations between MOA and TIP for the four operators ranged from 9.8 to 24.1 for MOA_o and 16.0 to 27.5 for MOA_p.

The agreement between the calculated difference of 81 μmol/L by Kramer and Tessier (1982), their measured difference of 82 μmol/L, and our measured value of 89 μmol/L for standardized $Ca(HCO_3)_2$ solutions provides support for a difference between MOA and Gran alkalinity of approximately 80 μmol/L assuming (1) the pink endpoint is used, (2) the endpoint occurs at or near pH 4.0, and (3) natural dilute lake waters mimic prepared bicarbonate solutions. However, the MOA titration provides for endpoints other than 4.0 (APHA 1971). Clark (1927) and Kolthoff (1931) show a color change for methyl orange starting at pH 4.4 and extending down to pH 3.1. The 6th edition of Standard Methods for alkalinity (a copy of which was obtained from the Trout Lake Laboratory), which presumably was used by the early investigators, states that the sample should be titrated to a definite color change (APHA 1925). This lack of explicit direction allowed for considerable interpretation of endpoints.

As a check on whether the methyl orange indicator affected the sample alkalinity, we measured 10 pairs of samples with and without the methyl orange indicator, ranging in concentration from 0 to 126 μmol/L. The difference of only 1 μmol/L was not significant, $P \leq 0.05$. Although methyl orange indicator itself has a pH of 4.2, the potential effect of the indicator is diluted by using only 3 to 4 drops per 100 mL of sample. To evaluate these possible MOA endpoints for Wisconsin lake water, we titrated samples containing methyl orange indicator with the pH electrode and recorded the first significant color change. Two technicians, operating independently, each observed an orange endpoint near pH 4.6 and a pink endpoint at pH 4.0. In addition, both operators detected a very faint pink color near pH 4.3, which is close to the endpoints of pH 4.4 also selected by Lewis (1982) and Burns et al. (1981). These differences in endpoints for MOA titrations are expected, considering the color changes observed throughout a wide pH range; the problem is further compounded by the presence of organic material in lake waters.

The historical records show that there was some dissatisfaction with the MOA endpoint used by the WGNHS during the 1930's (University of Wisconsin Archives). In a letter written to Chauncey Juday, Lowell Taylor explains and interprets the experiments he had conducted during the summer of 1932 regarding alkalinity titrations. Taylor worked as a chemist at the Trout Lake Laboratory for several years, and judging from entries in the laboratory notebooks, was very familiar with the analytical methods of the survey and the chemistry of the study lakes. Taylor, suspecting that the MOA method overestimated true alkalinity, experimented with different corrections for MOA using titrations with methyl orange indicator in distilled water. Taylor recommended to Juday that the bias in the MOA be corrected by subtracting 1.5 mg/L bound CO_2 from the existing titrations (equal to an alkalinity bias of 68 μmol/L). Juday apparently decided against incorporating Taylor's recommendation because the tabulated and published data are not corrected for this difference.

We attempted to resolve the question of which MOA endpoint was used by examining the relationship between conductivity, calcium, and alkalinity in the WGNHS data and comparing the relationship with that observed in the modern data set. Implicit in this approach is the assumption that the pCO_2, dissolved organic carbon concentrations, and metals such as aluminum in the lakes have not changed. A comparison of the historical and recent color measurements for all 149 lakes showed that present color values were generally 30% lower than the historical measurements (recent color = 2.7 (± 1.9) + 0.7 (± 0.03) historical color, $r^2 = 0.80\%$, $s = 18$ PCU). However, evaluation of the published historical method (Juday and Birge 1933) and examination of photographs showing the historical measurement procedure suggests that differences in methodology probably account for the observed differences between historical and modern color. The lakes in the study area are primarily calcium bicarbonate systems; consequently, they show strong agreement between conductivity and alkalinity in the recent data set, with a correlation coefficient of 0.99 (excluding 27 of 149 lakes showing evidence of being impacted by road salt). If we assume that an increase in lake water alkalinity would result in a proportional increase in conductivity, then we should observe no increase in alkalinity for those lakes showing no increase in conductivity. Therefore, the difference between historical and recent alkalinity measurements for lakes showing no change in conductivity should be a result of methodological differences, i.e, it should be equal to the difference between MOA endpoint used historically and the TIP method employed today. Note that the converse of this assumption (i.e., an increase in conductivity will result in an increased alkalinity) is not valid because of the addition of other salts such as NaCl. We defined "no change" in conductivity for this test to represent a change of ≤ 2 μS/cm, which is within the accuracy of the method. This is one-half the value for the 95% conductivity confidence interval for a significant difference based on pooled standard deviations of the historical and recent data sets. A regression equation for predicting present TIP alkalinity from present conductivity for those lakes with no change in conductivity yields the following equation:

(4) TIP Alkalinity, μmol/L =
$-122\ (\pm\ 8) + 11.5\ (\pm\ 0.3)$ Recent Conductivity, μS/cm (@ 20°C)

$n = 45,\ r^2 = 0.98,\ P < 0.05,\ s = 32$

A corresponding regression equation for these same lakes using historical conductivity data determined for this lake data set (i.e., those lakes showing a conductivity change ≤ 2 μS/cm), yields the following equation:

(5) Methyl Orange Alkalinity, μmol/L =
$-68\ (\pm 8) + 11.6(\pm 0.2)$ Historical Conductivity, μS/cm (@ 20°C)

$n = 45,\ r^2 = 0.98,\ P < 0.05,\ s = 34$

Because the slopes in equations 4 and 5 are not significantly different, we can combine the recent and historical data to determine the difference between the MOA and TIP alkalinity. The difference between the alkalinity methods is represented by the value of the indicator or dummy variable (see Snedecor and Cochran 1967, p. 416) of 57 μmol/L.

(6) Combined Alkalinity, μmol/L =
$-181\ (\pm 7) + 57\ (\pm 7)\ X_1 + 11.6\ (\pm\ 0.2)$
Combined Conductivity, μS/cm (@ 20°C)
$n = 90,\ r^2 = 0.98,\ P < 0.05,\ s = 33.$

This approach indicates that the historical MOA endpoint for the WGNHS data corresponds to a pH of about 4.17. This value is higher than the value of 4.04 determined by Kramer and Tessier (1982) and closer to values of 4.3 observed in our laboratory tests and the endpoints of 4.25 selected by Asbury et

al. (1989), 4.4 selected by Lewis (1982) , and 4.38 selected by Burns et al. (1981). A similar regression approach for predicting the difference between MOA and TIP using calcium could not be accomplished because of significant differences in slopes between the historical and recent data sets (1.67 and 1.40, respectively).

Our attempts to resolve the MOA bias for the WGNHS data fall into two groups: (a) those which assume an MOA endpoint, and (b) those which assume that certain components of the historical and recent chemical composition (e.g., pCO_2, weak acids, and metals) of a given lake are unchanged. The latter methods, which will be discussed later, include a nonlinear model relating pH to alkalinity, dissolved CO_2, and weak acid contribution.

The differences in endpoints are expected, considering that any endpoints within a range might be acceptable as defined by the method. The WGNHS technicians employed standardization practices by titrating to a color reference solution. Color distinctions are subjective, consequently requiring color standards, and a large degree of variability and error with the method should be expected among different analysts. It is likely that the WGNHS MOA values represent titrations to a range of endpoints, depending upon the care taken by the analyst in reproducing the color reference standards and the variability introduced by the observer for the colored samples. Without additional evidence to resolve this problem, we have elected to use 57 μmol/L derived by our analysis to correct for the historical alkalinity measurements of Wisconsin lakes, a value close to that recommended by L. Taylor (WGNHS chemist) and intermediate between those used by Kramer and Tessier (1982) and Lewis (1982). This will most likely result in unavoidable over- and underestimations of the historical alkalinity for individual lakes, but we believe it best represents this group of Wisconsin lakes.

Calcium — The accuracy reported for the historical calcium determinations was considered acceptable and no adjustment was made to these values. Relationships of historical calcium with conductivity and alkalinity show different intercepts and slopes compared to relationships among these three variables in the recent data perhaps indicating the need to correct historical calcium measurements, however no basis is available for making a correction. As noted, there may be a slight positive bias in the recent calcium values, but the magnitude of this possible bias (3 μmol/L) does not warrant adjusting the recent data. There were historical and recent calcium measurements for 116 of the 149 lakes.

Hydrogen ion (pH) — The majority of the WGNHS pH measurements is colorimetric (all pre-1932 data and an unknown percentage of 1932–41 data), whereas the recent pH data are available for both potentiometric and colorimetric methods on the same samples. The options we have for comparing pH from the two time periods are to compare (1) historical colorimetric to recent colorimetric, (2) historical colorimetric to recent potentiometric, and (3) the limited data on known historical potentiometric pH to recent potentiometric. Several comparisons of historical pH measurement have not been adjusted for differences between colorimetric and potentiometric methods (Hendrey et al. 1980; Burns et al. 1981; Lewis 1982). Schofield (1982) adjusted Hellige colorimetric measurements to potentiometric pH using a regression equation ($r^2 = 0.96$). Kramer and Tessier (1982) illustrate some of the errors associated with colorimetric methods and propose a method of correcting for these errors, assuming one knows which indicator was used.

We examined the relationship between colorimetric (LaMotte) and potentiometric pH for 316 samples from 59 lakes collected in 1980. The overall fit (Fig. 1a) appears reasonably good ($r^2 = 0.87$). For a subset of these recent samples ($n = 175$), we recorded the type of indicator used for the colorimetric pH measurements. The indicators and their respective Pearson correlation coefficients with potentiometric pH are: phenol red (0.83), bromthymol blue (0.58), bromcresol purple (0.75), and bromcresol green (0.91).

A comparison of the WGNHS unpublished colorimetric and potentiometric pH measured by L. Taylor during 1932 again shows good overall agreement between two methods ($r^2 = 0.85$). However, the agreement between colorimetric and potentiometric pH for a given indicator is in some cases poor: phenol red ($r^2 = 0.74$), chlorphenol red ($r^2 = 0.11$), bromthymol blue ($r^2 = 0.38$), and bromcresol purple ($r^2 = 0.09$). Not only do the individual indicator relationships exhibit fairly weak linearity (with the exception of phenol red), but the slopes of the regressions are significantly greater than one and different in most cases from one another (Fig. 1b). It is worth noting, however, that the r^2 for each indicator depends on the relative magnitude of the accuracy and applicable pH range. For example, if two indicators yield equally accurate results but one covers a pH range larger than the other, then the former will yield a greater coefficient of determination. Detailed evaluations of the problems measuring colorimetric pH at low ionic strength are presented by Clark (1927) and Kolthoff (1931).

Kramer and Tessier (1982) demonstrated the effect a color indicator can exert on a softwater sample by adding bromthymol blue indicator and observing the increase in potentiometric pH over 1 unit. Although this is an extreme example (the authors added bromthymol blue, which has a minimum range of pH 6.0 to a sample with a pH of 5.2), it serves to caution against direct use of colorimetric pH without some knowledge of the limitations of the indicators in extremely soft water. We also observed similar effects of colorimetric indicators on potentiometric pH for sample waters with low ionic strength and for waters in which a large difference exists between the sample pH and the pK of the indicator. The shift in potentiometric pH caused by the colorimetric indicators is greatest between pH 5 and 6 (Fig. 1c). Consequently, for those lake waters that we suspect are most likely to be impacted by acidic deposition (e.g., alkalinity < 40 μmol/L), the probability for large errors in colorimetric pH is greater than for the higher alkalinity lakes.

The problem of colorimetric pH measurement error must have been especially great when measuring the pH of previously unsampled lakes, as the investigators did not have the benefit of previous lake data. Although wide-range pH indicators were available, they still suffered from the same problem in very dilute waters where the pK of the indicator can mask the true pH of the sample. An example of this type of error, which we observed in our recent (1980) data, was the pH determination for Sugar Camp Lake. In measurements of samples for which the lake identity was unknown to the technician, this lake consistently showed a potentiometric pH for the surface waters between 5.2 and 5.4 ($n = 10$), yet the colorimetric pH values ranged from 5.0 (bromcresol green) to 6.7 (bromthymol blue). Conversely, it is also possible to err on the low side with colorimetric indicators. This type of error appears to have been responsible for a large number of low pH observations during the summer of 1930. A check of pH outliers in the historical

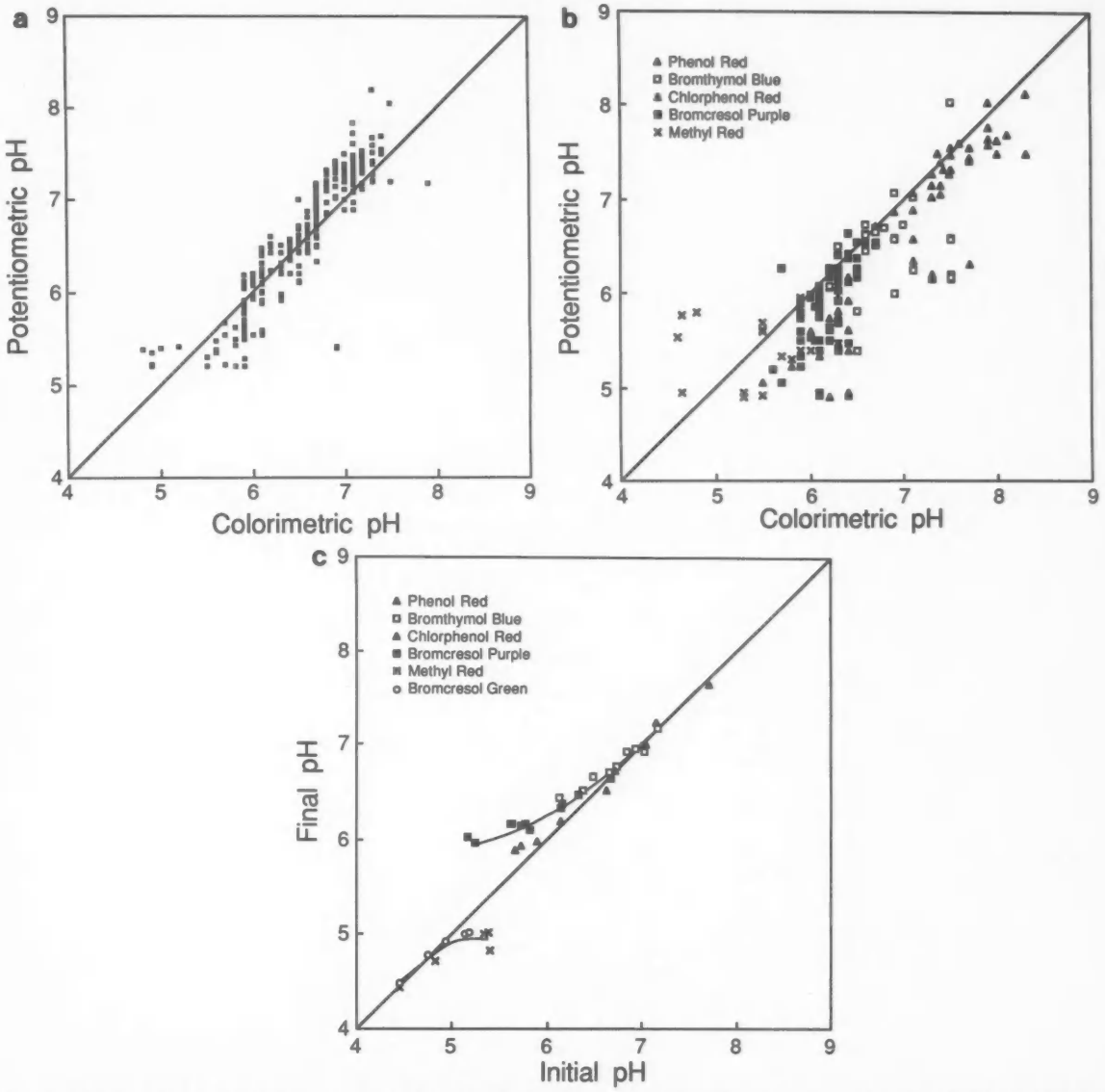

FIG. 1.(a) Recent potentiometric pH versus recent colorimetric (LaMotte) pH for northern Wisconsin lake samples. The solid line is the line of equality. The best fit overall regression model is slope 1.153 (± 0.025) and intercept −0.907 (± 0.166), $n = 314$, $r^2 = 0.87$, $P < 0.01$; (b) Historic potentiometric pH (quinhydrone electrode) versus historic colorimetric pH for five indicators. Regression parameters for individual colorimetric indicators are: bromthymol blue (slope 1.43 (± 0.98), $r^2 = 0.74$, $n = 75$, $P < 0.05$), bromcresol purple (slope 1.67 (± 0.18), $r^2 = 0.61$, $n = 56$, $P < 0.05$), bromcresol green (too few points), and phenol red (slope 0.874 (± 0.167), $r^2 = 0.46$, $P < 0.05$); (c) Effect of color indicators on pH of lake water for six indicators and their respective specified range of operation: bromthymol blue (6.0–7.4), bromcresol purple (5.2–6.6), bromcresol green (4.0–5.4), phenol red (6.8–8.2), chlorphenol red (5.4–6.8), methyl red (10 drops, 4.4–5.8).

study set for lakes with multiple observations showed 24 highly suspicious values (e.g., ⩾ 1.0 pH unit from the individual lake mean), only 1 of which was higher than the mean. Of the remaining 23 outliers on the low side, all but 1 occurred in 1930 and only 1 of these was ouside the pH range 5.0 to 5.5. Such a large number of abnormally low values in these lakes strongly suggested operator error in selecting the appropriate pH indicator for the more dilute lakes.

Suspect historical pH and alkalinity data were excluded by fitting the data against a nonlinear model developed for the modern data similar to that used by Small and Sutton (1986). For a given color range, the values of the pCO_2 and weak acid concentration were adjusted to best fit the modern pH-alkalinity data where pH = f (alkalinity, pCO_2, [weak acid]). These fits were achieved using five classes of apparent color, 0 ⩽ 2.5, 2.5 ⩽ 10.0, 10 ⩽ 20, 20 ⩽ 60, and > 60 PCU. The resulting

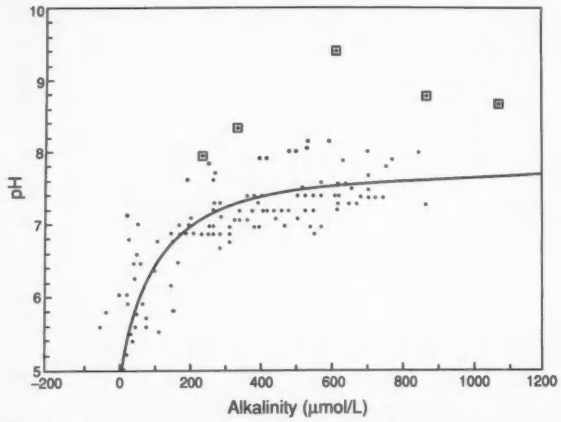

FIG. 2. An example of rejection of historical observations (squares) based on inconsistencies in pH and alkalinity. The curve represents a best-fit nonlinear model that corresponds to a pCO_2 of 3.03 and a weak acid concentration of 81 μmol/L. This example is for lakes with present color from 60 PCU; disassociation constant for weak acids was assumed to be pK 6.0 based on results of fitting modern observations.

pCO_2 values corresponding to these ranges of color were 3.37, 3.33, 3.31, 3.13, and 3.03, respectively. After determining the appropriate model parameters for each color range using modern color data, the historical data (historical color adjusted to correspond with modern color by multiplying historical values by 0.7; see earlier discussion of color measurements), were adjusted for the best fit by allowing a bias to be computed for alkalinity and pH. In this approach, it was assumed that the lakes experienced no systematic change in pCO_2 or weak acid concentrations. Outliers, defined here as those observations 2 SD from the fitted curve, were identified and removed from the set (e.g. Fig. 2).

The computed bias in pH and alkalinity derived from the model-fitting process (Table 1) indicates that the historical pH measurements underestimated true pH, whereas MOA alkalinity overestimated true alkalinity. The clearwater lakes (color ≤ 2.5 PCU) show the least bias in alkalinity, roughly one-half that observed for the colored lakes (color > 20 PCU). Three factors may contribute to this observation: (1) the color of the darkwater lakes may make it more difficult to observe a color change in the methyl orange indicator, (2) the organic compounds in the colored lakes may provide additional buffering to the system, and (3) the bias in the MOA may not be additive, which means that the alkalinity bias cannot be corrected using a constant.

If the alkalinity bias is multiplicative (i.e., the bias is a function of the alkalinity concentration), lakes with higher alkalinity will exhibit a higher bias. Because lakes in northern Wisconsin with higher alkalinity are generally also those with higher color, it is conceivable that the bias is independent of lake color. Regardless of how the historical data are partitioned, historical pH and alkalinity measurements show substantial bias. When averaged across color ranges, the model fitting approach yields a negative pH bias of 0.31 units and a positive alkalinity bias of 57 μmol/L for the raw historical data. These are 0.36 units and 53 μmol/L, respectively, after rejecting outliers identified in the first run. The agreement in alkalinity correction factor of 57 μmol/L between the combined regression analysis approach and this independent nonlinear model fitting approach lends support for the use of 57 μmol/L as the MOA correction factor for this data set.

Methods of Presenting the Most Representative Lake Chemistry for Lakes with Multiple Observations

We examined the variability of surface water chemistry by repeated sampling of 60 lakes in the summer of 1980; one lake was later excluded from the comparison. Fifteen of the lakes were sampled weekly for 10 consecutive weeks while the remaining lakes were sampled three times. At each lake visit, the surface (~ 0.5 m) water was sampled in replicate at each of three locations, forming an equilateral triangle near the center of the lake. The variances of the pH, alkalinity, and conductivity across the three stations were generally much less than the variances across time, indicating that precise location on a lake (within the pelagic zone) probably had little effect on the results. However, some of the lakes showed considerable variation across the summer, particularly in pH. These large fluctuations appeared to be related to photosynthetic activity as indicated by chlorophyll *a* measurements. Individual lake estimates of variability are difficult and costly to obtain; consequently, most general comparisons, including this study, rely on the use of pooled estimates. We did, however, calculate indi-

TABLE 1. Bias in historical pH and alkalinity measurements computed using a nonlinear model for raw and screened data.

		Computed bias					
		pH		alkalinity (μmol/L)		Historical observations	
Modern color range (PCU)	n[a]	raw[b]	screened[c]	raw	screened	Rejected	Retained
≤ 2.5	156	−0.44	−0.49	+36	+32	9	147
2.5 ≤ 10	196	−0.35	−0.38	+51	+47	13	183
10 ≤ 20	341	−0.23	−0.27	+59	+57	16	325
20 ≤ 60	254	−0.22	−0.29	+74	+71	15	239
> 60	130	−0.33	−0.38	+63	+58	5	125
Total	1077					58	1019

[a]Using data from the summers of 1925–31 only. Post-1931 pH data were both colorimetric and potentiometric; consequently the computed bias for pH in the later period may differ from pre-1931 measurements.
[b]Using raw historical observations.
[c]After omitting outliers.

vidual lake estimates of variability for those lakes repeatedly sampled in 1980, and compared the results of analyzing the changes in pH with all analyses resulting from pooled estimates. There were only minor differences in the confidence intervals for the three variables for those 59 lakes in which an individual variable was significantly less or greater than the pooled estimate (Eilers et al. unpublished data).

The averages for all parameters represent a simple arithmetic average. It has been common practice to calculate average pH values of lake samples by converting pH values to $[H^+]$, averaging the $[H^+]$, and then reconverting the $[H^+]$ average to pH. At first examination, this approach appears to be correct, since pH units are logarithmic and it is commonly accepted that in making comparisons one should not average log units. Furthermore, it is absolutely necessary to convert pH to $[H^+]$ when computing mass balances such as $[H^+]$ deposition. However, when comparing lake water pH from different dates, it is more appropriate to provide an estimate of the most probable pH. By converting pH values to $[H^+]$ one places more emphasis on the low pH values for a given lake and hence introduces a negative bias in the average pH (Bartlett 1982). Also, because pH is measured in the logarithmic scale, errors in measurement are generally normally distributed in this scale. Average pH is thus a valid statistical method for determining the most probable pH. A more complete discussion of this problem is provided by Boutilier and Shelton (1980).

Land Use Development

Shoreline development, defined as the presence of permanent dwellings within approximately 100 m from the lake shore, was used as one factor to evaluate changes in lake chemistry. The number of dwellings present during the historical lake survey was available from state Land Economic Surveys conducted during the 1930's. Recent dwelling counts were made from contemporary USGS topographic maps supplemented by aerial photographs. The median number of shoreline dwellings per lake in the historical period was 4, compared to 26 per lake in the recent period. Thirty-seven lakes in the historical period lacked any shoreline dwelling compared to only 16 lakes in the recent period. For purposes of distinguishing between degrees of increases in shoreline development, we defined three groups of lakes: (1) undeveloped; those lakes with a maximum of one dwelling in either the historical or recent period; (2) moderately developed and (3) highly developed lakes. These were defined on the basis of the recent dwellings normalized by lake area. The highly developed group of lakes defined on this basis had a median of 33.5 dwellings per lake.

Results

The lakes in this study were selected in a non-random manner, which can cause concern about how well the "found" sample reflects the population of lakes implicitly represented by the study. The final set of 149 study lakes, although selected on the basis of data availability and consistency, has chemistry similar to that of population estimates based on the statistical probability sample from EPA's Eastern Lake Survey (Table 2; Linthurst et al. 1986). This latter survey sampled north-central Wisconsin, a subregion that agrees reasonably well with the area of lakes selected for this historical comparison. An estimated 41% of the lakes in the study area had ANC $\leq$ 50 μeq/L, based on the Eastern Lake Survey, whereas 30% of the lakes in this study group had ANC $\leq$ 50 μeq/L, (based on the recent data). We believe that the findings from this comparison of historical lake chemistry are generally applicable to other lakes in northern Wisconsin, although the lakes from this study appear to be somewhat more alkaline than a random sample of lakes in this area.

TABLE 2. Mean (and median) present surface water chemistry from this study and from the Eastern Lake Survey.

Variable	This study, Recent Data (n = 149)	Eastern Lake Survey (n = 155, $\hat{N}$ = 1510)[a]
pH[b]	7.03 (7.12)	6.61 (6.67)
Alkalinity (μmol/L)	296 (168)	289 (104)
Calcium (μmol/L)	128 (74)	110 (51)
Conductivity[c]	45.0 (29.0)	40.9 (25.3)

[a]Data from Subregion 2C (Northcentral Wisconsin) where n = sample size; $\hat{N}$ = estimated population of lakes (source: Kanciruk et al. 1986).
[b]Open system pH.
[c]Corrected to 20°C and 25°C, respectively.

Comparison of Historical Versus Recent Measurements

Some indication of variability in the lake chemical data, as well as variability in the changes taking place, can be seen in plots of recent measurements versus historical measurements (Fig. 3). Here, the expected relationship would follow directly along the diagonals in Fig. 3, with the scatter of points on each side being a qualitative measure of lake or sample variability (seasonal or diurnal), differences in analytical methods, or systematic change over time. A bias to one side or the other suggests either a systematic difference in measurements associated with historical versus recent methods or a systematic trend toward elevation or depression in the recent values compared with historical data.

As indicated earlier, conductivity is one variable for which measurement methods have not changed appreciably, but Fig. 3a shows a systematic change toward higher values in the recent measurements. Apparently, most lakes have experienced small to relatively large increases in this variable. Of course, any one of several factors, including application of road salt, cultural eutrophication, changes in hydrology, or ion addition from atmospheric inputs all contribute to the observed effect. Indeed, 27 lakes had the combination of (1) major increases in conductivity, (2) increases in calcium, and (3) a significant length of paved roadway that is maintained in the winter; these lakes are suspected of having been influenced by runoff containing both $CaCl_2$ and NaCl from the deicing chemicals. When these sites are removed, the resulting regression is more tightly aggregated (r^2 = 0.98), has a slope of 1.03, and has an intercept of 3.5 μS/cm 20°C), s = 3.9.

The scatter on both sides of the diagonal for alkalinity and calcium (Fig. 3b and 3c) may represent random error or a pattern in which some lakes have decreased in the recent alkalinity value, while others have increased. Both are possible, because of the opposing effects of forest aggradation versus logging on soil CO_2 concentrations, and other human influences. Calcium, like alkalinity, appears to have increased across a wide range of concentrations. Indeed, the slope of the regression line (1.13) and the intercept (28 μmol/L) suggests that the higher the his-

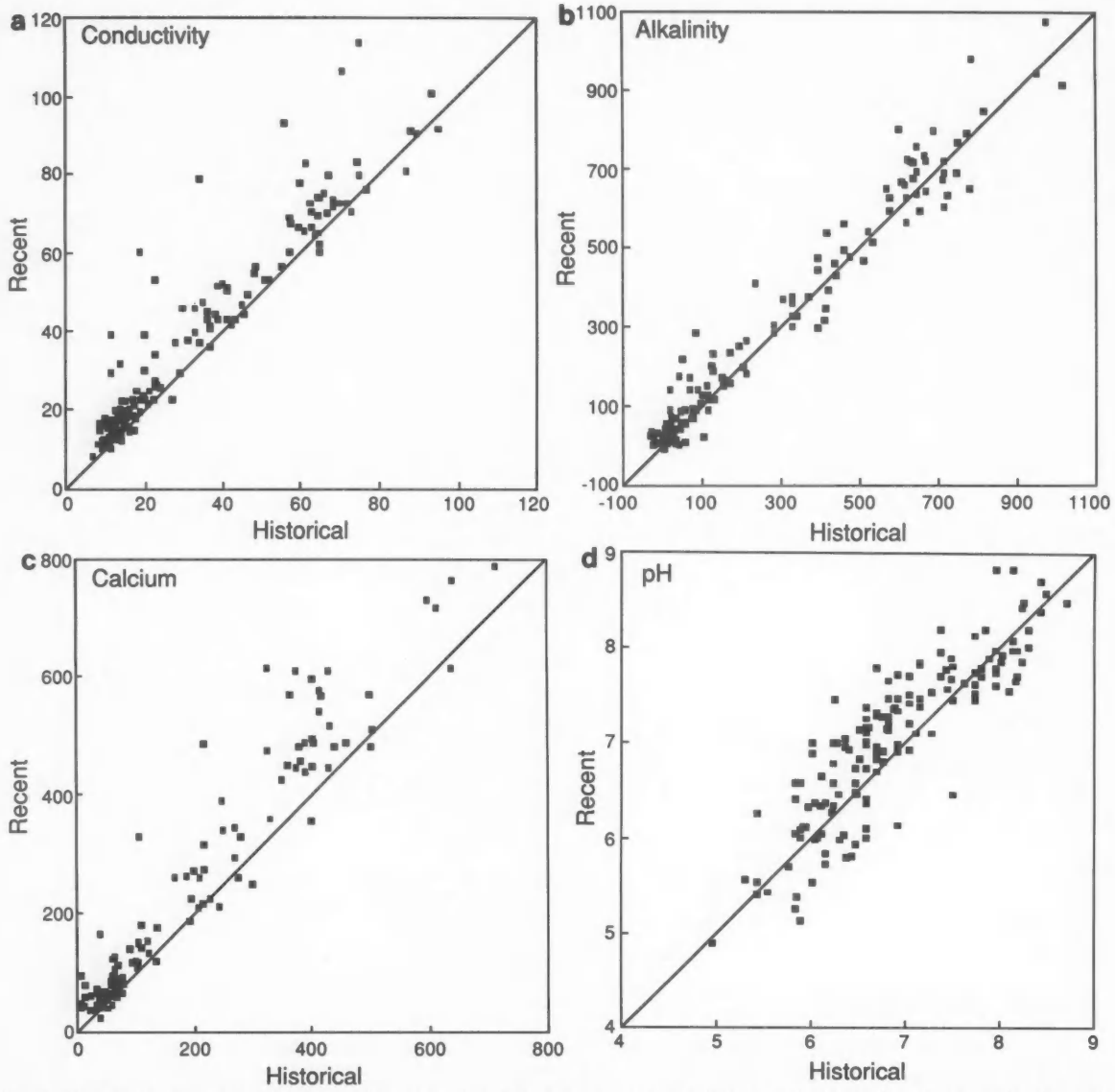

FIG. 3. Water quality changes in 149 northern Wisconsin Lakes. (a) Conductivity, μS/cm at 20°C; (b) Alkalinity, where 57 μmol/L is subtracted from historical values; (c) Calcium, μmol/L; (d) pH, where historical values have been adjusted using this regression equation (pH = 1.19–1.17 [historical]).

torical calcium concentration in the water, the larger has been the increase in calcium.

The broadest scatter of all is in the pH measurements (Fig. 3d). This is not unexpected, given the difficulties in resolving differences between potentiometric and colorimetric measurements, the high temporal variability, and the inherent problems of measuring pH in dilute solutions. The relatively large number of depressed pH values in the low pH range could be, in part, the consequence of methodological differences. The large number of sites showing recent increases in pH across the middle range is consistent with the increases in calcium and in alkalinity, but this also may reflect, in part, a systematic underestimation of pH in the historical measurements. The decline in the pH values for historically high pH lakes could result from unusual biological activity at the time of historic sampling, or, more likely, from methodological differences; however, these uncertainties will not greatly affect an interpretation concerned primarily with changes in softwater lakes. Although there is considerable uncertainty in this comparison of pH, a comparison of recent pH measured with comparable indicators and historical colorimetric pH values also shows that pH increased in 49 of the 59 lakes sampled in 1980.

Average Responses

Overall, small to moderate increases in average conductivity, alkalinity, calcium, and pH occurred between the historical and

TABLE 3. Changes in conductivity, alkalinity, calcium, and pH for Northern Wisconsin Lakes[a]

Case	n[b]	Category	Conductivity (μS/cm @ 20°C)			Alkalinity (μmol/L)			Calcium (μmol/L)			pH		
			Historical	Recent	Change	Historical	Recent	Change	Historical	Recent	Change	Historical	Recent	Change
I	149	All lakes (Median)	22.5	29.0	+3.4	125	168	+14	55	76	+16	6.76	7.12	+0.18
		(Mean ± SE)[c]	33.1 (1.9)	38.7 (2.1)	+5.6** (0.7)	267 (23)	286 (23)	+19** (5)	99 (8)	124 (9)	+25** (3)	6.88 (0.007)	7.03 (0.07)	+0.16** (0.03)
		(Range)	7.0:95.0	8.2:113.5	−6.7:44.5	−30:1017	−7:1075	−138:197	4:357	13:393	−25:145	4.97:8.72	4.90:8.84	−1.06:1.19
II	45	"No" change	16.3	16.3	+0.5	58	63	+7	30	34	+13	6.59	6.88	+0.18
		in conductivity	27.4 (3.2)	27.8 (3.2)	+0.4** (0.1)	193 (37)	198 (37)	+5 (5)	67 (12)	84 (13)	+16** (4)	6.71 (0.11)	6.86 (0.12)	+0.15* (0.06)
			7.0:89.8	8.2:90.1	−1.8:2.0	−15:951	−7:939	−94:111	4:307	13:357	−25:133	5.43:8.32	5.13:8.83	−1.06:1.08
III	19	Undeveloped watersheds	14.3	14.7	+0.5	22	29	+10	23	32	+7	6.13	6.27	+0.05
			14.2 (1.0)	15.3 (1.1)	+1.0 (0.5)	43 (13)	62 (14)	+19* (8)	28 (4)	36 (4)	+8** (2)	6.27 (0.09)	6.35 (0.14)	+0.08 (0.10)
			7.0:22.5	8.2:24.5	−1.8:7.8	−12:213	−7:186	−35:129	6:54	13:75	−7:22	5.86:7.05	5.13:7.45	−0.77:1.18
IV	42	Developed watersheds	31.5	44.3	+6.4	221	281	+18	134	137	+20	6.82	7.18	+0.16
			38.1 (3.8)	47.7 (4.3)	+9.6** (1.8)	316 (44)	348 (46)	+32** (10)	131 (18)	163 (18)	+32** (8)	7.02 (0.12)	7.19 (0.12)	+0.17** (0.06)
			9.0:88.0	13.2:113.5	−6.7:41.0	−30:817	2:980	−94:196	7:321	20:382	−25:145	5.32:8.72	5.54:8.84	−0.53:1.08

[a]Changes are reported as present minus historical value. Alkalinity is adjusted by subtracting 57 μmol/L from the historical values. Historical pH values are colorimetric values transformed to potentiometric values by the equation potentiometric pH = −0.907 + 1.153 (colorimetric pH). Averages and standard errors are calculated from 149 single values (either single data points or averages) per lake.

[b]Number of samples for calcium are I, 116; II, 39; III, 18; and IV, 31.

[c]Probability levels are indicated at $P < 0.01$ (**) and $P > 0.05$ (*).

modern period (Table 3). Averages for two subsets of the lakes, undeveloped and developed watersheds, suggest that much of the increase in conductivity and calcium can be attributed directly to cultural development of lakeshores and watersheds. The increase in calcium for the developed lakes is four times that of the undeveloped lakes. Striking increases in lake conductivity for some lakes appear to have resulted from the use of road salt, which began for this area in 1957 (B. Davies, Vilas Co. Hwy. Dep., 1982, pers. comm.). An evaluation of conductivity for lakes in the region using data from county surface resources publications (Black et al. 1963; Andrews and Threinen 1966) indicates that the lakes in the area had experienced little increase in conductivity by the early 1960's. However, data from this study show that conductivity increased over 100% in one lake and over 200% in another, both of which are bordered by highways but are otherwise undeveloped.

In view of the possible importance of summer residence "development," some additional explanation is required. Most lakes in the area have a substantial number of summer residences and readily qualify as developed; only a few lakes, mostly small and isolated, have no development. At the same time, historical changes in local land cultivation, regrowth of the forest following burning, and recent logging near a few lakes are not reflected in this simple grouping. Of the 19 watersheds classified as undeveloped, only one of the lakes shows a significant ($P < 0.05$) increase in conductivity, whereas 91 of 123 developed lakes show statistically significant increases.

As observed for conductivity, all groups of lakes show average increases in alkalinity. Two partially overlapping groups of lakes, those showing no increase in conductivity and those without development, both show an apparent increase in alkalinity, but substantially smaller in the former than the latter. Indeed, both developed and undeveloped lakes show the same average change, but the undeveloped group includes all 11 lakes that had a significant ($P < 0.05$) decline in alkalinity.

The average increases in pH for all lakes are relatively consistent with the observed increases in alkalinity and conductivity. One anomaly, however, is the pH increase in the lakes showing no increase in conductivity and a relatively small average increase in alkalinity (6 μmol/L). The group of lakes showing the greatest decrease in acidity, based on change in $[H^+]$, is the undeveloped set; this group historically had more acidic lakes than the developed set. At the same time, this underdeveloped group has the largest proportion of lakes (~ 35%) that show a decline in pH. Interpretations of these averages across large and diverse groupings of lakes ultimately must be tempered by analysis of changes on an individual-lake basis.

Some lakes are less acidic and more saline than they were 50 yr ago (Table 4). The two lakes with statistically significant decreases in pH do not show concomitant decreases in alkalinity, and of the 46 lakes with significant and insignificant decreases in alkalinity, only 16 have corresponding decreases in pH. Contingency analysis of the increases and decreases among the four variables shows an association between changes in alkalinity and conductivity, but comparisons between other variables were not significant, suggesting an overall independence in the changes in lake chemistry. Obviously pH and alkalinity values in surface waters are not independent, but are closely related chemically. Lack of a strong association between the changes in pH and alkalinity may reflect high uncertainty associated with the historical measurements for pH.

TABLE 4. Number of lakes with significant and insignificant ($P<0.05$) changes in conductivity, alkalinity, calcium, and pH.

	95% Confidence[a] interval	Decreases		Increases	
		Significant	Insignificant	Significant	Insignificant
Conductivity (μS/cm @ 20°C)	3.98	3	17	67	62
Alkalinity (μmol/L)	54.8	11	35	28	75
Calcium (μmol/L) (n = 116)	28.5	0	16	39	61
pH (stand. units)	1.07	0	55	2	92

[a]Confidence intervals were based on an analysis of the 59 lakes with repeated samples in the summer of 1980. The 95% confidence limits were calculated from the sum of the pooled variances of the historical and present sampling periods.

Discussion

When this study was initiated, the questions that we posed were:

(1) can the historical data be used to evaluate changes in water quality?

(2) has the chemistry of the lakes changed? and

(3) can these changes be attributed to natural or anthropogenic factors?

We believe that the historical data are generally internally consistent and well correlated with present lake chemistry, thus affirmatively answering our first question about the quality of the historical data. The unscreened historical and recent data (Eilers et al. 1983b) for 161 lakes were also made available to the National Academy of Sciences. These studies indicate that historical pH and alkalinity measures from Wisconsin are adequate to evaluate changes in water chemistry. Kramer et al. (1986) noted that the magnitude of corrections for the historical pH values ranged from +0.5 to −1.25 pH units and that the likely endpoint for historical alkalinity ranged from pH 4.19 to 4.04. Andren and Bischoff (1985) suggest corrections of similar magnitudes. The smaller range in suggested correction values for the historical alkalinity values compared to those for pH support our interpretation that greater confidence can be placed in the alkalinity data. Unlike the Adirondack lakes for which selection of a methyl orange alkalinity endpoint is critical to the interpretation (e.g., Kramer et al. 1986; Asbury et al. 1989), the evaluation of historical change in Wisconsin lakes is relatively insensitive to reasonable variations in titration endpoints. For example, if we had used a correction of 68 μmol/L suggested by Taylor (corresponding to a pH endpoint of about 4.11) to compensate for the overtitration of historical alkalinity, the number of significant decreases in alkalinity would have changed from 11 to 9 lakes.

The changes in lake chemistry (Tables 3 and 4) demonstrate that there have been increases in conductivity, alkalinity, calcium, and pH. Furthermore, the relative magnitude of the increases in conductivity, alkalinity, and pH are generally consistent among these three variables. The calcium increases for cases I, II, and IV (Table 3), however, appear to be greater than expected, given the increases in conductivity and alkalinity. Examination of case II shows an increase in calcium of 16.5 μmol/L, which conflicts with the lack of change in conductivity. On average, calcium increases over 2.5X that of alkalinity (on an equivalent basis), suggesting cation mobilization, possibly coupled with bicarbonate neutralization. However, the increases in these constituents are clearly not uniform among all lakes. Comparison of lakes in cases II-IV with all lakes (case I), reveals some important differences in the lake changes. The undeveloped lakes show no statistically significant change in conductivity, but do show an increase of 1 μS/cm in conductivity, which is 60% of that expected for an increase of 19 μmol/L in mean alkalinity. Although the highly developed lakes (case IV) show an even greater increase in alkalinity than the undeveloped lakes (case III), the relative increase in alkalinity for the developed lakes is actually less than in undeveloped lakes. The latter group also exhibits an apparent increase in calcium of 7–8 μmol/L, yet the calcium increase in the highly developed lakes of 32 μmol/L represents a four-fold increase. The contrast between the relatively small changes in the undeveloped and highly developed watersheds suggests two important findings: (1) lack of a major change in the undeveloped watersheds indicates that atmospheric deposition has not significantly changed the chemistry of the study lakes in the last 50 yr within the errors of this analysis, and (2) the large chemical increases in the highly developed lakes implicate land use practices as a major influence on changing water quality of the area.

These principal findings must be tempered by two concerns. First, limitations in the historical data, although compensated to some degree, remain problematic. The quality of the historical data varies by parameter and can be ranked such that our confidence in individual observations decreases as follows: conductivity >>alkalinity > calcium >> pH. Unlike the other variables, the uncertainty in alkalinity is caused by both analytical uncertainties (e.g., uncertainty in titration endpoints), and because substantial changes in alkalinity can be masked by either decreases in organic acids or increases in metals such as aluminum. For example, alkalinity may be defined as the excess of cations over strong acid anions where:

$$\text{Alkalinity} = \Sigma \text{ cations} - \Sigma \text{ strong acid anions}$$

$$= (2[Ca^{2+}] + 2[Mg^{2+}] + [Na^{+}] + [K^{+}] + [Al^{n+}] + [Fe^{n+}] + [Mn^{n+}]).$$

$$([NO_3^{-}] + 2[SO_4^{2-}] + [Cl^{-}] + [F^{-}])$$

The presence of organic anions results in a negative bias in ANC titration results, as compared to the above definition (Sullivan et al. 1989). Under this definition, organic anions could be precipitated as organo-metallic complexes with little change in alkalinity. For example, Schafron and Driscoll (1987) reported a reduction in concentrations of dissolved organic carbon for an Adirondack lake that was associated with the formation of alumino-organic solutes. Chemical changes of this type can leave the water more transparent, yet more toxic to fish.

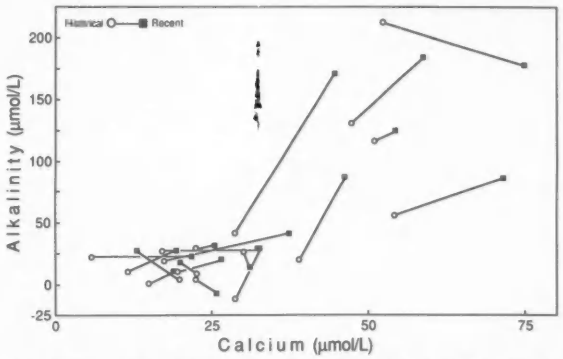

FIG. 4. Changes in calcium and alkalinity (historical alkalinity adjusted by −57 μmol/L) for 18 undeveloped lakes in northern Wisconsin with both historical and recent calcium and alkalinity values. Historical values are shown as open circles; recent values are shown as closed squares.

A second major uncertainty in the analysis is the causality of the changes. The contrast between the responses for the undeveloped and highly developed lakes strongly implicates lake shore development as an important contributing factor to many of the changes. There is considerable evidence from other research that lake shore development promotes increased input of minerals and nutrients to a lake that would have the effect reported here. One problem with the association of lakeshore development and water quality change is that the increase is confounded by hydrologic lake type. Of the 19 undeveloped lakes, 17 are seepage lakes. Because development is more common adjacent to drainage lakes that are typically more alkaline (Eilers et al. 1983a), it is difficult to ascribe the change unequivocally to lake shore development. Also, many other changes occurred in the region during the intervening 50 yr. These additional changes include: (1) major regrowth of the forest cover, (2) dramatic reduction in forest fires, (3) changing SO_x and NO_x emissions, (4) differences in precipitation, and (5) increase in fish stocking and other lake management activities.

Although complete testing of the relationship of each factor to lake change is beyond the scope of this analysis, it is possible to place some of these factors in perspective. Some of the changes are easily associated with a causal factor. For example, the large increases in conductivity exhibited by 27 lakes are clearly linked to the use of de-icing chemicals. For some lakes showing major increases in development, we observe increases in all parameters that could reasonably be attributed to cultural eutrophication. Twenty-one lakes undeveloped in the 1930's experienced increases in the number of shoreline dwellings from 1 to as many as 54; the median increase in the number of shoreline dwellings for all 149 lakes was 20. The 42 highly developed lakes (case IV) experienced increases in alkalinity and calcium twice as great as the remaining 107 study lakes. Although cultural development is implicated as an important factor in chemical changes in many of the study lakes, it is surprising that a comparison of historical and recent Secchi disk values (historical values increased by 5% to account for differences in Secchi disk diameter) reveals no significant differences.

In several watersheds where cultural development has not occurred, substantial increases in chemical constituents were apparently associated with fires that burned a major portion of the watersheds (based on records from the Wisc. Dept. Nat. Resour., Woodruff). Rangeline Lake and Frank Lake are examples of undeveloped watersheds where increases in alkalinity from 12 to 41 μmol/L and from 35 to 171 μmol/L were apparently associated with major fires in their watersheds within the decade preceding the recent surveys.

However, there are few obvious explanations for the chemical changes in the remaining lakes. One could relax the criteria for defining developed lakes and perhaps account for additional lakes that have become culturally enriched. However, the chemical changes for the undeveloped lakes and for the light-to-moderately developed lakes become indistinct.

Another watershed factor that could have a profound influence on lake chemistry is forest regrowth. Most of the virgin white pine forest once covering the study area was completely removed by logging and fires from 1880 to 1920 (Alhgren and Alhgren 1983). The white pine was largely replaced by mixed deciduous forest type, dominated by aspen which was in the early stages of regrowth during the historical lake survey. Replacement of the white pine with deciduous species may have had a major influence on the hydrologic cycle, because the latter species are associated with deeper snowpacks and lower amounts of evapotranspiration. Urie (1967) observed increased groundwater storage under mixed hardwoods compared to pine plantations in a similar hydrologic setting in Michigan. An increase in the groundwater level could have increased the hydraulic gradient, thus increasing the groundwater contribution to the lakes. The importance of groundwater contributions to a softwater seepage lake in the study area was demonstrated by Lin and Schnoor (1986), where the groundwater contributed 3% of the hydrologic input to Vandercook Lake, yet accounted for 24% of the lake alkalinity budget. Lakes that have experienced an increase in both calcium and alkalinity (in the absence of shoreline development) may be reflecting increased groundwater inputs. For several of these undeveloped lakes, concomitant increases in alkalinity and calcium (Fig. 4) are evident. Although one could observe the same response resulting from a change in the chemical composition of the groundwater (Anderson and Bowser 1986), this latter explanation appears unlikely, given the depth of the till in the area and high buffering capacity of the aquifers.

An alternative mechanism for increasing the contribution of groundwater inputs is through increased precipitation. Increased groundwater input to the lakes resulting from an elevated water table could also have resulted from differences in precipitation before and during the respective study periods. Analysis of annual precipitation indicates that from 1917–1937 Wisconsin experienced an extended drought period, with 1923 and 1930 being particularly dry years (Zaporozec 1980). A below-average precipitation period of shorter duration occurred from 1974 to 1976, the latter year having the severest drought on record. However, the precipitation in Wisconsin during the 1970's (including the primary data collection years in the recent lake survey 1979–80), still was above average. Confirmation that the precipitation patterns observed statewide also occurred in the study area was made by evaluating U.S. Dep. of Commerce precipitation records from Rhinelander and other climatological stations in the study area. An analysis of annual precipitation during the historical and recent study periods showed no significant ($P < 0.05$) difference between the periods. When reanalyzed on a seasonal and monthly basis, the recent period had significantly greater precipitation during the summer months. Perhaps of greater importance to the lake and groundwater levels

is the influence of annual precipitation for the several years preceding the lake surveys.

The majority of the lakes show no statistically significant change in the four parameters shown in Table 4. For the small number of lakes that may have decreased in alkalinity and pH, we were interested in determining whether there was an association, if any, with acidic deposition. Recent information on historical emissions of sulfur and nitrogen (Gschwandtner et al. 1985; Johnson 1986) indicates that sulfate deposition may have changed little in Wisconsin between the two study periods and may actually have decreased when considered on a regional basis, assuming that SO_4^{2-} deposition is directly proportional to SO_2 emissions. This assumption is subject to question given the increase in average stack height of the emission sources. A comparison of the historical and recent concentrations of lake sulfate indicates an increase of approximately 5 μmol/L. Nitrogen deposition appeared to have increased several fold and may have acted as an alkalizing factor (see Kilham 1982), presuming that the current ratio of NO_3^-/NH_4^+ was also greater than one. Therefore, if sulfate deposition remained constant or declined and nitrate deposition increased, it would appear that some lakes may have responded by becoming more alkaline. This group of lakes would include those that show an increase in alkalinity without a corresponding increase in calcium. However, this type of chemical response also could be mimicked by fertilization derived from cultural development.

Paleolimnological analyses of nine northern Wisconsin lakes are consistent with a slight acidification of the lakes starting from about 1870 to 1900. Four of the lakes examined showed decreases in pH of about 0.3 units, two lakes showed slight increases, and three lakes showed no significant change (Charles et al. 1989). A recent review of the acid-base status of lakes in the Upper Midwest suggests that acidification has been slight (~ 10 μmol/L) for the most sensitive lakes (Cook and Jager 1989). The magnitude of the loss of alkalinity in the Upper Midwest estimated by Cook and Jager (1989) equals (on an equivalence basis) the estimated increase in SO_4^{2-} for these Wisconsin lakes. If the loss of alkalinity in Wisconsin lakes is associated with replacement by SO_4^{2-}, then lakes showing statistically significant losses of alkalinity during the last 50 yr would be associated with factors in addition to a relatively small acidic deposition signal.

Examination of factors leading to changes in lake chemistry have focused on changes in lake inputs. Another possibility is that some of the lakes have altered as a consequence of within-lake changes in biota. Wisconsin has a long history of active fisheries management (Becker 1983). From the late 1800's to the late 1930's fish were gathered from backwaters on the Mississippi River and other rivers in the state and transported to lakes in northern Wisconsin. This practice supplemented an active fish stocking program begun in the late 1800's. By 1937 Wisconsin established a national record for the propagation and distribution of fish (Wisconsin Conservation Department 1963). Common fisheries practices since the 1940's have included management of the lakes for predator species (e.g., muskellunge, northern pike, walleye, largemouth bass, and trout) with a de-emphasis of the centrarchids native to the lakes. It is conceivable that these widespread biomanipulations have resulted in corresponding shifts in dominant zooplankton and phytoplankton species, which in turn may have altered lake chemistry (Shapiro et al. 1982). An examination of state records (Wisc. Dep. Nat. Resourc., Woodruff, Rhinelander) for 59 lakes sampled in 1980 showed that at least 57 of these had received some form of fish management practice (most commonly, fish stocking).

The chemical record of lakes in northern Wisconsin can be used in a variety of ways to examine changes in lake chemistry over a 50-yr period. The most striking chemical change evident in many of the lakes is an increase in salinity. For those lakes showing statistically significant changes, the evidence strongly supports an alkalinization of lakes associated with cultural enrichment of the lakes from shoreline development. Other factors such as forest fires can account for a smaller number of changes. Most of the remaining lakes show relatively small increases or decreases in alkalinity that, assuming the methodological differences are adequately treated, can likely be attributed to multiple factors, including forest maturation and changes in sulfur and nitrogen emissions. Considering the magnitude and character of the changes that have occurred in northern Wisconsin lakes, it appears that land use changes have the greatest potential for contributing to further changes in the lakes. The large confidence bounds for any single lake computed on the multiple observations in the recent data further suggest that major biological changes would occur in these lakes before statistically reliable changes in lake chemistry could be documented. Given the modest acidic deposition in northern Wisconsin, uncertainty in historical methods, and the multitude of counteracting natural and anthropogenic influences, we cannot attribute the changes in lakewater chemistry to acidic deposition alone.

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A Genetic Comparison of Sympatric Populations of Sand Lance (Genus *Ammodytes*) from the Region East of Cape Soya, Japan

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Okamoto, H. 1989. A genetic comparison of sympatric populations of sand lance (genus *Ammodytes*) from the region east of Cape Soya, Japan. Can. J. Fish. Aquat. Sci. 46:1945–1951.

Sand lance (Genus *Ammodytes*) collected from four stations off Japan and one station at Kodiak, Alaska were genetically characterized at 17 protein coding loci using starch-gel electrophoresis. Sand lance in Wakkanai (Cape Soya, Japan) consist of two genetically distinct groups. They are fixed for different alleles at four loci (*Ldh-2, -3, G3pdh-2,* and *Mdhp-2*). The genetic structure of one of the groups (Wakkanai-a group, W-a) is similar to that of *A. personatus* around Japan. The other group (Wakkanai-b group, W-b) has different genetic structure from either *A. personatus* or the Alaskan collection, which is presumed to belong to *A. hexapterus*. It is not presently possible to identify the affiliation of the W-b group; however, despite its sympatry with the W-a group, it is reproductively isolated and therefore is probably a distinct species occurring northeast of Hokkaido.

Nous avons procédé à la caractérisation génétique de lançons (genre *Ammodytes*) recueillis à quatre stations situées au large des côtes du Japon et à une station située à Kodiak, en Alaska. Nous avons étudié 17 loci codant pour des protéines à l'aide d'un appareil d'électrophorèse sur gel d'amidon. Les lançons recueillis à Wakkanai (cap Soya, Japon) comportaient deux groupes génétiques distincts. Ces deux groupes ont des allèles différents en quatre loci (*Ldh-2, -3, G3pdh-2,* et *Mdhp-2*). La structure génétique de l'un de ces groupes (groupe Wakkanai-a, W-a) est similaire à celle de la population d'*A. personatus* présente dans la région du Japon. La structure génétique de l'autre groupe (groupe Wakkanai-b, W-b) diffère de celle d'*A. personatus* et de celle du groupe de lançons recueillis en Alaska qui appartiendraient présumément à l'espèce *A. hexapterus*. Il n'est pas possible pour l'instant d'identifier à l'espèce le groupe W-b; cependant, même si les populations des groupes W-a et W-b sont sympatriques, le groupe W-b reste isolé au plan de la reproduction, ce qui indique qu'il s'agit probablement d'une espèce distincte vivant au nord-est de l'île d'Hokkaido.

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Sand lance (Genus *Ammodytes*) are common in coastal waters and offshore banks in the temperate to arctic North Pacific, including its marginal seas, the Sea of Japan, Okhotsk Sea, Bering Sea and Chukchi Sea. Two species of this genus are distributed in the North Pacific Ocean (Lindberg 1937; Matsubara and Ochiai 1965), *Ammodytes personatus*, which is found in the coastal region around Japan, and *A. hexapterus*, which is distributed farther north. There is, however, uncertainty and confusion in the designation of *Ammodytes* species in the North Pacific (Andriashev 1954; Kawamura 1940; Hashimoto 1984) as well as in the North Atlantic Ocean (Richards et al. 1963; Reay 1970). This confusion partly results from the overlapping ranges of meristic characteristics among species (Auster and Stewart 1986; Winters and Dalley 1988). Since meristic characters are known to be variably affected by environmental temperature (Tåning 1952; Garside 1966), it may be difficult to use such characters to classify species which have as wide a latitudinal distribution as sand lances. Genetic characters which are not affected by environmental factors such as temperature have promise for solving taxonomic problems in the genus *Ammodytes*.

Kitaguchi (1979) indicated that east of Cape Soya, there coexist two distinctive sand lance groups which have modal differences in anal fin ray and vertebral numbers. He suggested that one group belongs to *A. personatus* and the other belongs to *A. hexapterus*. It is difficult to distinguish these two groups at the individual level because of overlap in these meristic characters, and this makes it difficult to understand their taxonomic and ecological relationships. Iwata, Kitaguchi, and Numachi (pers. comm., Ocean Research Institute, University of Tokyo, Minamidai, Nakano-Ku, Tokyo, Japan), found that the sand lance from east of Cape Soya in summer had different alleles of *Ldh-B, G3pdh, Sod,* and *Mdhp-B* loci from those of *A. personatus* inhabiting northern Japan. They presumed, without comparison to sand lance from far northern and eastern Pacific, that the fish might be *A. hexapterus*.

In a previous report (Okamoto et al. 1988), the genetic structures of ten sand lance populations around Japan were surveyed. This work did not include the Wakkanai (Cape Soya) collections reported here. This paper compares the genetic structure of two groups of sand lance collected from east of Cape Soya, and discusses the genetic relationships between them, and with populations of sand lance around Japan, and an Alaskan group.

Materials and Methods

Sand lance were sampled from four stations in Japan (Ise Bay, Rishiri, Kushiro and Wakkanai) and one station in Alaska (Kodiak, Fig. 1). Samples near Wakkanai were captured east of Cape Soya (45°33′50″ N, 142°19′00″E) in May 1987. Spec-

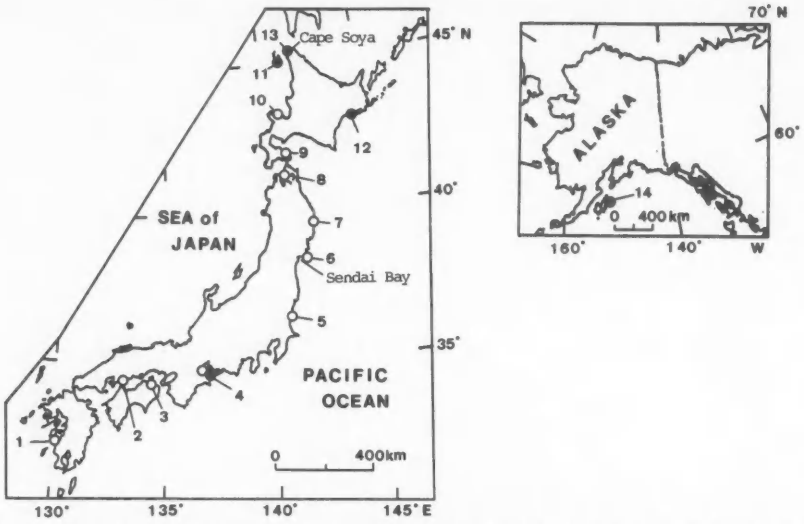

FIG. 1. Sites of collection for sand lance from Japan (left) and Alaska. Open circles are sites whose populations were analyzed in our previous study (Okamoto et al. 1988) based on 10 loci. Closed circles are sites analyzed in this study based on 17 loci. 1 = Ushibuka, 2 = Onomichi, 3 = Takamatsu, 4 = Ise Bay, 5 = Hitachi, 6 = Amiji Is. 7 = Ryogoku Bay, 8 = Kusodomari, 9 = Usujiri, 10 = Yoichi, 11 = Rishiri, 12 = Kushiro, 13 = Wakkanai, 14 = Kodiak, Alaska.

TABLE 1. Protein coding loci, their Enzyme Commission (EC) numbers and designations, and the tissues and buffers in which they were resolved. Buffers used are numbered as in the text.

Enzyme	EC No.	Designation	Tissue	Buffer
Creatine kinase	2.7.3.2	*Ck-2*	Muscle	I
Fumarate dehydrogenase	4.2.1.2	*Fh*	Muscle	I
Glyceraldehyde-phosphate dehydrogenase	1.2.1.12	*Gapdh-1*	Eye	I
		Gapdh-2	Heart	I
Glycerol-3-phosphate dehydrogenase	1.1.1.8	*G3pdh-1*	Liver	II
		G3pdh-2	Muscle	II
Isocitrate dehydrogenase	1.1.1.42	*Idh-1*	Muscle	I
		Idh-2	Liver	I
Lactate dehydrogenase	1.1.1.27	*Ldh-1*	Eye	I
		Ldh-2	Muscle	I
		Ldh-3	Eye	I
Malate dehydrogenase	1.1.1.37	*Mdh-1*	Muscle	I
		Mdh-2	Liver	I
Malic enzyme	1.1.1.40	*Mdhp-2*	Muscle	II
Phosphoglucomutase	5.4.2.2	*Pgm*	Muscle	II
Phosphogluconate dehydrogenase	1.1.1.44	*Pgd*	Liver	I
Superoxide dismutase	1.15.1.1	*Sod*	Liver	III

imens were captured with dip net or trawl net, and carried to the laboratory packed in dry ice and stored at −20°C until analysed by electrophoresis. Eyes, hearts, liver and skeletal muscle were removed from frozen fish and minced in distilled water. The soluble components from minced frozen tissues were absorbed on a filter paper wick (Toyo Roshi Co. No. 51A).

Seventeen presumptive loci encoding eleven enzymes were analyzed by starch-gel electrophoresis. The buffers used for electrophoresis were I, citrate-*N*(3-aminopropyle) diethanol amine buffer at pH 6.9 (Clayton and Tretiak 1972), II, tris-citrate buffer at pH 8.0 (Clayton and Gee 1969), and III, discontinuous tris-citric acid (Ridgway et al. 1970). Starch-gel plates were made with 12% starch (Nakarai Chemicals, LTD. Lot No. M7N1805). Electrophoresis for resolution of *Sod* (Table 1) was carried out at 4°C, 4mA·cm^{-2} for about 2 h using the buffer system of Ridgway et al. (1970), and for 3 h for other enzymes in the other buffers. The staining procedure followed Shaw and Prasad (1970) and Harris and Hopkinson (1976), with slight modifications.

Chi-square goodness-of-fit was used to compare observed frequencies of phenotypic classes with those expected under Hardy–Weinberg equilibrium. Homogeneity of allelic frequencies was examined using log-likelihood ratios (*G*-test) (Sokal and Rohlf 1981).

Genetic similarity and distance (Nei 1972) were calculated between all possible pairs of collections surveyed in this study, and between fifteen collections including nine collections previously studied (Okamoto et al. 1988). Dendrograms (UPGMA; Sneath and Sokal 1973) were used to express the relationships between sand lance populations.

TABLE 2. Observed(obs) and expected(exp) genotypic distributions at three polymorphic loci (*Ldh-2*, *Pgm*, *Sod*) in sand lance collection of Wakkanai. Chi-square values and probabilities of deviations from expected Hardy-Weinberg distributions are also given.

Locus	*N*	Frequency of '100' allele	Genotypic distributions				chi-square	*P*
Ldh-2	100	0.56	100/100	100/300	300/300			
			obs 55	1	44		96.01	<<0.005
			exp 30.8	49.4	19.8			
Pgm	100	0.66	100/100	100/183	183/183	other		
			obs 57	18	24	1	34.25	<<0.005
			exp 43.6	44.2	11.2	1.0		
Sod	100	0.56	70/70	70/100	100/100			
			obs 35	18	47		40.29	<<0.005
			exp 19.4	49.3	31.4			

TABLE 3. Allele frequencies observed at 10 polymorphic loci in six sand lance collections.

		Collections					
Locus	Alleles	Ise Bay	Rishiri	Kushiro	Kodiak	W-a	W-b
Ldh-2	100	1.000	1.000	0	1.000	1.000	0
	300	0	0	1.000	0	0	1.000
	N	75	80	70	30	55	44
Ldh-3	100	1.000	1.000	0	1.000	1.000	0
	250	0	0	1.000	0	0	1.000
	N	75	80	70	30	55	44
Mdh-1	100	1.000	1.000	1.000	0	1.000	1.000
	112	0	0	0	1.000	0	0
	N	75	80	70	30	55	44
Idh-2	100	1.000	0.981	0	0	0.982	0
	120	0	0	0.625	0.400	0	0.670
	140	0	0.019	0.375	0.600	0.018	0.330
	N	75	80	30	10	55	44
Mdhp-2	82	0	0.675	0	0	0.627	0
	100	1.000	0.288	0	0	0.309	0
	132	0	0.038	0	0	0.055	0
	147	0	0	0	0	0.009	0
	171	0	0	1.000	1.000	0	1.000
	N	75	80	70	30	55	44
G3pdh-2	39	0	0	0	0	0.018	0
	44	0	0	0.071	0	0	0.080
	100	1.000	0.725	0	0	0.655	0
	122	0	0	0.821	0	0	0.852
	178	0	0.275	0	0	0.327	0
	200	0	0	0.107	1.000	0	0.068
	N	75	80	70	30	55	44
Gapdh-1	80	0	0.956	1.000	1.000	0.973	1.000
	100	1.000	0.044	0	0	0.027	0
	N	75	80	70	30	55	44
Pgdh	87	0	0.006	0.900	0.267	0.009	0.932
	100	1.000	0.994	0.100	0.733	0.991	0.068
	N	75	80	70	30	55	44
Pgm	100	1.000	0.994	0.314	0.167	0.991	0.250
	183	0	0.006	0.664	0.833	0.009	0.739
	283	0	0	0.021	0	0	0.011
	N	75	80	70	30	55	44
Sod	70	0	0.119	0.957	0	0.109	0.852
	100	1.000	0.881	0.043	1.000	0.891	0.148
	N	75	80	70	10	55	44

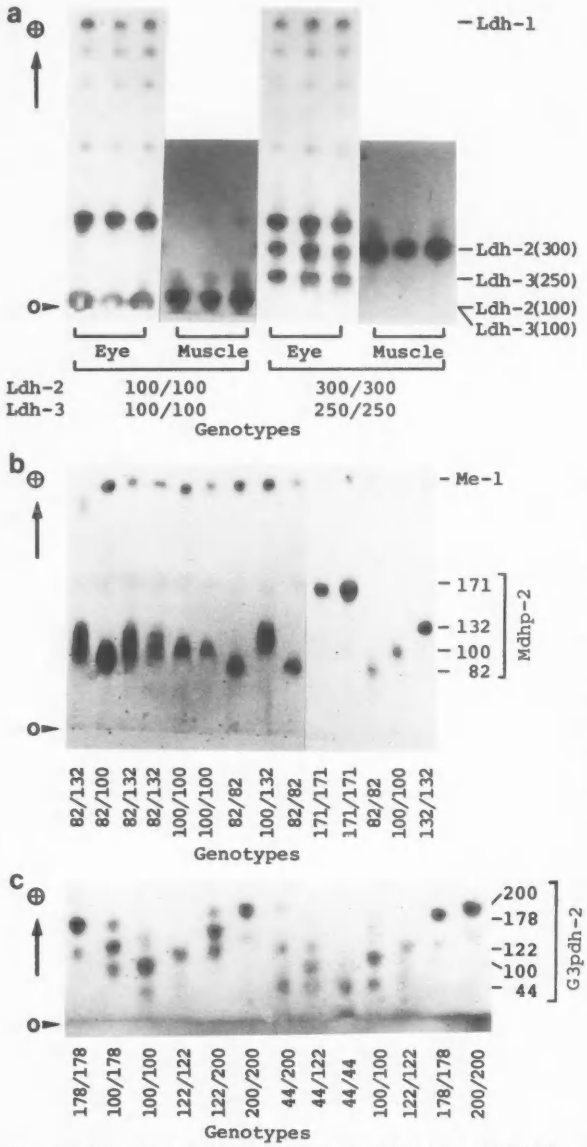

FIG. 2. Allelic variations at the *Ldh-2, -3* (a), *Mdhp-2* (b), and *G3pdh-2* (c) loci in sand lance from Wakkanai. Muscle tissue was used for *Mdhp-2* and *G3pdh-2* loci.

The number of vertebrae (including urostyle) was counted on soft X-ray images of samples from Wakkanai and Alaska.

Results

Among seventeen presumptive loci surveyed, nine loci were polymorphic in sand lance collection from Wakkanai. These were *Ldh-2, -3, Idh-2, Mdhp-2, G3pdh, Pgdh, Pgm, Sod* and *Gapdh-1*. However, frequencies of phenotypes at each locus except *Gapdh-1* were quite different from those expected under Hardy–Weinberg equilibrium. At *Ldh-2*, phenotypes 100/100 and 300/300 occurred in 55 and 44 individuals, respectively, in

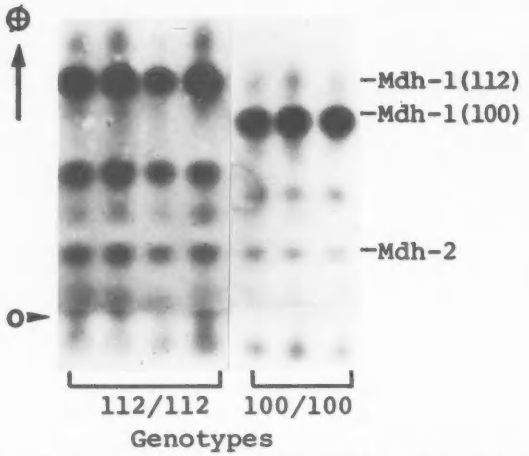

FIG. 3. Allelic variations at *Mdh-1* (liver) in sand lance from Wakkanai (right three individuals) and Kodiak (left four individuals).

a sample of 100 individuals, while *Ldh-2(100/300)* occurred only once (Table 2). Departure from Hardy–Weinberg expectation was also quite large for *Pgm* and *Sod*. The sand lance aggregation at this area apparently consisted of two groups; W-a group in which *Ldh-2(100)* was fixed, and W-b group, in which *Ldh-2(300)* was fixed. Each of the two groups distinguished by genotypes of *Ldh-2* was tested for conformance to Hardy–Weinberg equilibrium. Allele frequencies for eight polymorphic loci (not including *Gapdh-1*) were calculated separately for each group. In chi-square contingency tests, observed genotypic values for eight polymorphic loci in each group did not depart significantly from expectations.

The allele frequencies at 10 polymorphic loci for the collections surveyed, including the W-a and W-b groups, are shown in Table 3. At four loci (*Ldh-2, Ldh-3, Mdhp-2* and *G3pdh-2*), the W-a group was fixed for different alleles from those of the W-b group (Fig. 2). At four other loci (*Idh-2, Sod, Pgdh* and *Pgm*), obvious differences in allele frequency were observed between the two groups. The specimen observed to have *Ldh-2(100/300)*, was heterozygous at three other distinctive loci (ie, *Ldh-3(100/250), G3pdh-2(122/178)*, and *Mdhp-2(100/171)*.

The allele frequencies of the W-a group were similar to those of the Rishiri collection at all loci. These two collections had at least one allele in common with that of Ise Bay at all polymorphic loci, although the allele frequencies of the former two collections at *Mdhp-2, G3pdh-2* and *Gapdh-1* differed from those of Ise Bay. The allele frequencies of the W-b group were similar to those of the collection at Kushiro at all polymorphic loci. At the four loci for which the W-a and W-b groups were fixed for different alleles, the Kodiak collection shared alleles at two loci (*Ldh-2, Ldh-3*) with the W-a group, and shared alleles at the other two loci (*Mdhp-2* and *G3pdh-2*) with the W-b group. At the *Mdh-2* locus, the Kodiak collection was fixed for the allele *Mdh-2(112)* which was not observed in any other group (Fig. 3).

Genetic similarities (*I*) and distances (*D*) were calculated from allele frequencies at 17 loci (Table 4). Data from six collections in this study and from another nine collections in a previous study (Okamoto et al. 1988) were combined, and genetic similarities and distances among fifteen collections were calculated based on 10 loci (Table 5). The results of UPGMA

TABLE 4. Estimates of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among six sand lance collections surveyed in this study, based on 17 loci.

	Ise Bay	Rishiri	Kushiro	Kodiak	W-a	W-b
Ise Bay		0.9100	0.5210	0.6640	0.9084	0.5211
Rishiri	0.0943		0.6096	0.7428	0.9996	0.6081
Kushiro	0.6520	0.4949		0.6727	0.6118	0.9987
Kodiak	0.4094	0.2973	0.3964		0.7463	0.6782
W-a	0.0960	0.0004	0.4914	0.2926		0.6105
W-b	0.6518	0.4974	0.0013	0.3884	0.4935	

TABLE 5. Estimates of Nei's genetic identity (above diagonal) and genetic distance (below diagonal) among 15 collections of sand lance based on 10 loci including data from Okamoto et al. (1988).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. Ushibuka		1.000	1.000	1.000	1.000	1.000	0.945	0.945	0.932	0.936	0.941	0.361	0.539	0.942	0.366
2. Onomichi	0.000		1.000	1.000	1.000	1.000	0.945	0.945	0.932	0.936	0.941	0.361	0.537	0.942	0.366
3. Takamatsu	0.000	0.000		1.00	1.000	1.000	0.947	0.946	0.933	0.937	0.942	0.362	0.541	0.944	0.367
4. Ise Bay	0.000	0.000	0.000		1.000	1.000	0.945	0.945	0.932	0.936	0.941	0.936	0.537	0.942	0.365
5. Hitachi	0.000	0.000	0.000	0.000		1.000	0.950	0.950	0.937	0.941	0.946	0.367	0.542	0.947	0.372
6. Amiji Is.	0.000	0.000	0.000	0.000	0.000		0.946	0.946	0.933	0.937	0.942	0.364	0.540	0.943	0.369
7. Ryogoku Bay	0.056	0.057	0.055	0.056	0.051	0.056		0.998	0.998	0.998	0.999	0.388	0.562	0.998	0.392
8. Kusodomari	0.056	0.057	0.055	0.057	0.052	0.056	0.002		0.999	0.998	0.999	0.394	0.563	1.000	0.397
9. Usujiri	0.070	0.071	0.069	0.071	0.065	0.070	0.002	0.001		0.999	0.999	0.399	0.560	0.999	0.401
10. Yoichi	0.066	0.066	0.065	0.066	0.060	0.065	0.002	0.002	0.001		0.999	0.407	0.556	0.998	0.407
11. Rishiri	0.061	0.061	0.059	0.061	0.055	0.060	0.001	0.001	0.001	0.001		0.396	0.559	0.999	0.398
12. Kushiro	1.019	1.020	1.017	1.021	1.002	1.009	0.948	0.932	0.919	0.900	0.927		0.472	0.397	0.998
13. Kodiak	0.619	0.622	0.614	0.621	0.612	0.617	0.577	0.575	0.580	0.586	0.582	0.750		0.563	0.485
14. Wakkanai-a	0.059	0.060	0.058	0.060	0.055	0.059	0.002	0.000	0.001	0.002	0.001	0.924	0.574		0.400
15. Wakkanai-b	1.005	1.006	1.003	1.007	0.989	0.996	0.937	0.924	0.914	0.898	0.921	0.002	0.723	0.917	

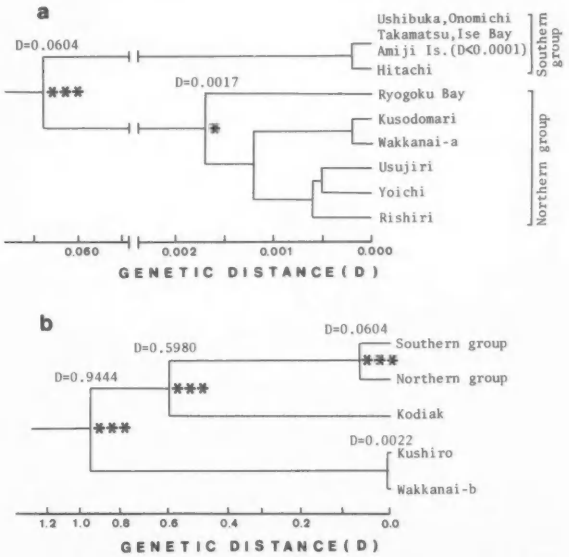

FIG. 4. Phenetic relationships among sand lance collections based on 10 loci. a, collections around Japan except W-b and Kushiro; b, all 15 collections surveyed. Asterisks denote junctions for which significant heterogenity (log-likelihood ratio) exists between the two branches (*$P<0.005$; ***$P<0.001$).

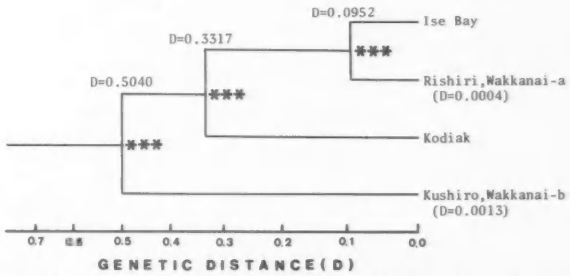

FIG. 5. Phenetic relationships among five collections of sand lance based on 17 loci. Asterisks denote junctions for which significant heterogeneity (log-likelihood ratio) exists between the two branches (***$P<0.001$).

cluster analysis based on genetic similarity between 15 collections for 10 loci, and between six collections for 17 loci are shown in Fig. 4 and Fig. 5, respectively.

The distribution of vertebral numbers in the complete Wakkanai sample was bimodal; one mode at 65 or 66 and another at 68 (Fig. 6a). After separation into W-a and W-b groups by genotypes of Ldh-2, the vertebral number was counted again for each group (Fig. 6b). The W-a group, which have *Ldh-2(100/100)*, showed one mode at 65 or 66 (range 64–68, $\bar{v}=65.7$), while the W-b group, which have *Ldh-2(300/300)* showed one mode at 68 (range 65–70, $\bar{v}=67.6$). The collection at Kodiak showed a mode at 69 (range 65–71, $\bar{v}=68.2$, Fig. 6c).

Discussion

The electrophoretic data clearly indicate the sympatry of two genetically distinct sand lance groups in the area east of Cape Soya. In a previous study, it was made clear that sand lance distributed around Japan consist of two groups which have different genetic structures, the northern and southern groups (Okamoto et al. 1988), separated at the latitude of Miyagi or

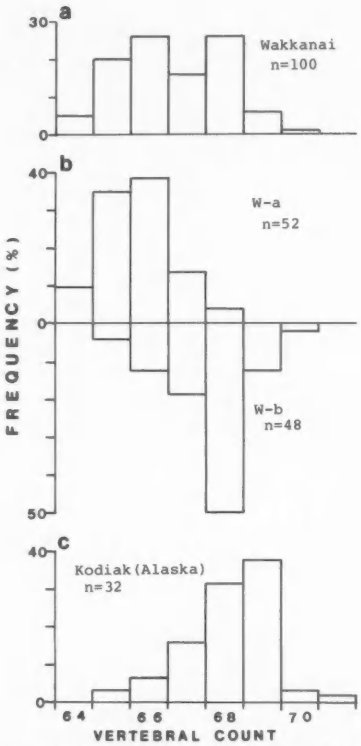

FIG. 6. Histograms of vertebral counts for collections of sand lance from Wakkanai and Kodiak.

Iwate Prefecture (38–39° N). The Wakanai-a (W-a) group and Rishiri collection cluster closely with four other northern collections (based on 10 loci, $D<0.0018$, Fig. 4a), while the Wakkanai-b (W-b) group clusters with the Kushiro collection ($D=0.0022$, Fig. 4b). The W-a group and Rishiri collection shared major alleles at similar frequencies with the northern group at all of these 10 loci (see Okamoto et al. 1988). The W-a and Rishiri collections probably belong to the northern group of *A. personatus*. Northern and southern groups shared at least one allele with each other at all loci surveyed (see Okamoto et al. 1988). Genetic distances were 0.0000-0.0003 (10 loci) among collections within the southern group, 0.0002-0.0025 (10 loci) among collections within the northern group, and 0.0604 (10 loci) or 0.0952 (17 loci) between these two groups. The distance between *A. personatus*, which consists of the northern and southern groups, and the W-b group including Kushiro was 0.9638 (10 loci) and 0.5441 (17 loci). These values are much higher than those observed between the northern and southern groups, which indicates that the W-b group is only distantly related to the northern and southern groups.

Despite sympatry of the W-a and W-b groups off northeastern Hokkaido, they were fixed for different alleles at four loci. The one specimen which was observed to have *Ldh-2(100/300)* genotype was also heterozygous at the other three distinctive loci of each group. This suggests that this individual was an F_1 hybrid between W-a and W-b individuals. These results suggest that strong reproductive isolation exists between these sympatric groups. It might be justified to treat these groups as different species. The group W-a apparently belongs to *A. personatus*, but to what species does the W-b group belong?

East of Cape Soya, Kitaguchi (1979) surveyed the meristic characters of sand lance and found two sympatric groups which have modal differences in vertebral and anal fin ray counts. He suggested that one group which had a mode in vertebral number (including urostyle) of 65 and 66 and a mode in anal fin ray number of 31, belongs to *A. personatus*, while another group which had a mode in vertebral number of 68 and a mode in anal fin ray number of 29 and 30, belongs to *A. hexapterus*. He indicated that the former, which is distributed around Japan, and the latter, which is distributed farther north, would overlap and coexist in this area at least in summer. In this study, the mode for vertebral number of the W-a group was consistent with Kitaguchi's *A. personatus* and the mode of the W-b group was consistent with *A. hexapterus*.

To confirm whether the W-b group belongs to *A. hexapterus* or not, the genetic structure of the collection from Kodiak, Alaska, was compared with that of the W-b group and *A. personatus* populations. Since *A. hexapterus* is distributed from southern California to Alaska and the Bering Sea, Chukchi Sea, Aleutian Islands, Okhotsk Sea, and Sea of Japan as far south as 44°N (reviewed in Hart 1973), Kodiak is near the center of distribution of this species. If the W-b group and Kodiak collection are from the same species, they should show a similar genetic structure.

A collection from Kodiak, however, showed different genetic structure from either the W-b group or *A. personatus* populations including the W-a group. Genetic distance between Kodiak group and W-b (including Kushiro), and *A. personatus* was 0.3924 (17 loci) and 0.3317 (17 loci), respectively. These values are similar in magnitude to that observed between Japanese *A. personatus* and the W-b groups. To establish the relationship between W-b and *A. hexapterus*, it would be necessary to survey the genetic structure of *A. hexapterus* populations extensively throughout its distribution. Assuming that the genetic relationship between the W-b group and the Kodiak collection reflects the relationship between W-b and *A. hexapterus*, the possibility arises that the W-b group may be a peculiar species occurring northeast of Hokkaido.

Hashimoto (1984) surveyed a number of meristic characters, fecundity and distribution of sand lance populations around Japan, and found that they were composed of three groups. Judging from their distribution, they correspond to the W-b group, the northern group, and the southern group, respectively. Hashimoto suggested the possibility of sub-specific relationships between these three groups because it was difficult to distinguish them by their external appearance. In this study, genetic distances (based on 17 loci) between Ise Bay (southern group) and the Rishiri and W-a populations (northern group) was 0.0952 (greater than the distance obtained previously between northern and southern groups based on 10 loci, $D=0.0612$). This value, 0.0952, which is intermediate between typical distances among congeneric species and distances among conspecific populations of fishes (Shaklee et al. 1982), may support Hashimoto's suggestion of subspecific status for southern and northern groups.

It is possible to identify two groups of sand lance distributed sympatrically northeast of Hokkaido at the individual level from their distinctive genotypes. For these groups, it should be possible to study morphological and ecological features in detail, and to discover the mechanisms that separate them.

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Absorption of Ascorbic Acid and Ascorbic Sulfate and their Interaction with Minerals in the Digestive Tract of Rainbow Trout (*Oncorhynchus mykiss*)

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Dabrowski, K., and G. Köck. 1989. Absorption of ascorbic acid and ascorbic sulfate and their interaction with minerals in the digestive tract of rainbow trout (*Oncorhynchus mykiss*). Can. J. Fish. Aquat. Sci. 46: 1952–1957.

The sites of absorption of ascorbic acid (AA) in the rainbow trout (*Oncorhynchus mykiss*) gut were located in the stomach (20.7%), pyloric caecae region (23.4%), middle intestine (21.9%) and posterior intestine (20.1%). Ascorbic sulfate (AS) were found to be absorbed in the stomach (27.7%) although it is likely that the figure obtained is an overestimate. This conclusion is based on the concentration factor of the intestinal contents (faeces/food ratio) being much higher for ascorbic sulfate than for an external dietary marker, chromic oxide. The apparent protein absorption rates from the three diets used were not significantly different ($P < 0.01$), ranging from 86.9 to 91.5%. Three subsequent samplings, one week apart, did not reveal changes in AA or AS absorption in trout, suggesting that fish were neither "saturated" with AA nor had they developed the means to utilize the dietary AS. In most cases, the mineral contents in faeces were similar for all diets, although the concentration factors (faeces/food level), varied greatly during the course of the trial (1.9–7.1 for Zn, 1.8–4.9 for Fe, and 2.4–3.7 for Cu). The concentrations of Fe and Zn in the contents of the posterior intestine were lower in the fish fed an AA-supplemented diet than in fish fed on AS and deficient diets, which may suggest an increased absorption of these elements.

Les auteurs ont montré que les sites d'absorption de l'acide ascorbique dans le tube digestif de la truite arc-en-ciel (*Onchorhynchus mykiss*) se répartissaient comme suit : estomac, 20,7 %, région de l'appendice pylorique, 23,4 %, intestin moyen, 21,9 % et intestin postérieur, 20,1 %. D'après certaines observations, le sulfate de l'acide ascorbique serait absorbé à 27,7 % dans l'estomac, bien qu'il s'agisse probablement d'une surestimation puisque le facteur de concentration du contenu intestinal (rapport fèces/aliments) est beaucoup plus élevé pour le sulfate de l'acide ascorbique que pour un marqueur alimentaire externe, l'oxyde de chrome. Les taux d'absorption apparente des protéines provenant des trois régimes utilisés n'étaient pas différents de façon significative ($p<0,01$) et variaient de 86,9 à 91,5 %. Trois échantillonnages subséquents, réalisés à une semaine d'intervalle, n'ont pas révélé de modification dans l'absorption par la truite de l'acide ascorbique ou du sulfate de l'acide ascorbique. Cela indiquerait que les poissons ne sont pas « saturés » en acide ascorbique ou qu'ils n'ont pas su utiliser le sulfate de l'acide ascorbique alimentaire. Dans la plupart des cas, les teneurs minérales des fèces étaient semblables pour les trois régimes, bien que les facteurs de concentration (rapport fèces/aliments) aient varié beaucoup au cours de l'étude (1,9 à 7,1 pour le Zn, 1,8 à 4,9 pour le Fe et 2,4 à 3,7 pour le Cu). Les concentrations de Fe et de Zn dans le contenu de l'intestin postérieur étaient plus faibles chez les poissons soumis à un régime avec acide ascorbique que chez les poissons soumis à un régime avec sulfate de l'acide ascorbique ou à un régime carencé; il y aurait donc pu avoir une absorption accrue de ces deux éléments.

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All cultured fish require ascorbic acid (AA) in their feeds. But, because of poor stability of ascorbic acid added to the diets, the chemically modified form, ascorbic acid sulfate (AS), has gained attention as the stable substitute with possibly similar biological potency as the original ascorbic acid. Contradictory results exist, however, concerning AS utilization and bioavailability in fish. On the one hand, Murai et al. (1978) with catfish and Soliman et al. (1986) with tilapia both demonstrated that prolonged feeding with AS led to a considerable decrease in tissue ascorbic acid as compared to controls fed with AA. Tucker and Halver (1984) and Tsujimura et al. (1978, 1981, 1982a) suggest on the other hand that AS can be utilized effectively by several fish species as a source of vitamin C.

The absorption of ascorbic acid has been assessed by several methods in higher vertebrates but not in fish. (1) Results obtained using in vitro techniques led to the conclusion that dehydroascorbic acid (DHA) is absorbed in the intestine by a Na-independent mechanism and that ascorbic acid (AA) is transported by a Na-dependent mechanism (Bianchi et al. 1986). (2) Studies on humans supported the hypothesis that ascorbic acid absorption is dose-dependent and that plasma concentration-time curves and urine excretion rate correspond to vitamin intake (Melethil et al. 1986). Both gastrointestinal absorption and renal reabsorption of ascorbate are saturable processes and therefore neither of them can be plotted in a linear

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fashion (Hornig et al. 1980). (3) Odumosu and Wilson (1978) used ligated segments of guinea pig intestine to elucidate the differences between the ascorbic acid transfer from the intestinal lumen into mucosa, muscle and blood in animals with various histories of vitamin C intake. (4) Oral application of ^{14}C ascorbic acid or its derivatives and measurement of radioactivity in the intestinal segments led Hornig et al. (1973) to the conclusion that vitamin C absorption sites are different in rat and guinea pig, being the ileum in the rat and the duodenum and proximal small intestine in the guinea pig. (5) An alternative approach, not used so far in vitamin C studies, is the examination of the ascorbic acid content in various parts of the digestive tract. This technique was used to study protein absorption in fish gut (Dabrowski 1986) and is a useful tool for comparing the absorption of vitamin C and its derivatives.

Since absorption of ascorbic acid has not been studied in fish in the past, the objectives of this study were: (1) to examine the absorption site of ascorbic acid and ascorbic sulfate in the digestive tract of the rainbow trout (*Oncorhynchus mykiss*, formerly *Salmo gairdneri*), and (2) to investigate the relationship between ascorbic acid and dietary minerals, Fe, Zn, and Cu, which are known to interact with ascorbic acid at the intestinal and the metabolic levels (Hornig et al. 1984).

Materials and Methods

The feeding experiment was conducted with rainbow trout yearlings of 50–70 g body weight. They were obtained from a local fish farm and kept for 2 wk in the laboratory prior to the experiment. They were fed a rainbow trout pelleted diet (Alma Futter, Kempten, F.R.G.), which contained 250 $\mu g \cdot g^{-1}$ of total ascorbic acid. At the start of the trial, fish were divided into groups of 10 individuals, and kept in tanks with several compartments, supplied with aerated city water, 10° ± 1.0°C. Each compartment had a volume of 250 L; dissolved oxygen was maintained close to saturation.

The diets were prepared by a formula similar to that used by Hughes et al. (1981) (Table 1). After mixing all dry ingredients, diets were supplemented with either 0 mg ascorbic acid$\cdot kg^{-1}$ (diet 1), 500 mg ascorbic acid$\cdot kg^{-1}$ (diet 2) or 1084 mg ascorbic sulfate (dipotassium ascorbic −2 sulfate dihydrate)$\cdot kg^{-1}$ (diet 3). Ascorbic acid (M.W. 176.1) and ascorbic sulfate (M.W. 381.8) were from F. Hoffmann-LaRoche, Basel, Switzerland. After mixing once more, lipid and water were added, and the diets were then processed on a laboratory pellet mill. Wet pellets were immediately frozen (−80°C) and freeze-dried within the next 24–48 h. The diets were stored in sealed bags in a freezer (−18°C) until required for feeding. Fish were fed 1.5% of their body weight per day.

Sample Collection and Biochemical Analysis

After 7, 14, and 21 d the fish were netted and anaesthetized with benzocaine and faeces were collected by squeezing the posterior part of the belly. Faeces collection took place at least 15 h after the last feeding.

At the termination of the feeding trial (28 d) fish were killed 16 h after feeding and the alimentary tract dissected into 5 parts as previously described (Dabrowski et al. 1986), i.e. stomach, pyloric caecae segment, mid-intestine and posterior intestine which was divided into two equal parts (see also Fig. 1). Samples from the different parts of the digestive tract of 5–6 fish per dietary treatment were squeezed out and pooled; they were kept on ice until further processed.

TABLE 1. Formulation of basal diet fed to rainbow trout.

Ingredient[a]	Concentration[b] (g/kg dry diet)
Casein	400
Gelatin	100
Dextrin	254.6
Carboxymethyl cellulose (CMC)	20
Soya oil[c]	60
Cod-liver oil[c]	60
L-methionine	2
L-tryptophan	0.4
Mineral mixture[d]	80
Vitamin mixture[e]	10
NaCl	5
Choline chloride	2
Chromic oxide	5

[a]Dietary ingredients were commercially obtained. The vitamin-free casein, gelatin, dextrin, CMC, BHT and free amino acids were from Sigma Chemical Co., St. Louis, MO, USA. Chromic oxide was from Merck, Darmstadt, F.R.G.

[b]The basal diet adds up to 999 g. The space is left for AA, AS or dextrin addition.

[c]Cod-liver oil and soya oil were from local commercial suppliers.

[d]The mineral supplement provided the following (g/kg of dry diet): $CaHPO_4 \cdot 2H_2O$ 18, $CaCO_3$ 19, KH_2PO_4 14, $NaHCO_3$ 8.88, $MnSO_4$ 0.35, $FeSO_4 \cdot 7H_2O$ 0.5, $MgSO_4$ 3, KIO_3 0.01, $CuSO_4 \cdot 5H_2O$ 0.03, $ZnCO_3$ 0.15, $CoCl_2 \cdot 6H_2O$ 0.002, $Na_2MoO_4 \cdot 2H_2O$ 0.008, $NaSeO_3$ 0.002.

[e]The vitamin supplement provided the following amounts per kg of dry diet: tocopheryl acetate 150 mg, biotin 5 mg, mesoinositol 400 mg, nicotinic acid 100 mg, thiamin HCl 30 mg, Ca-pantothenate 100 mg, folic acid 10 mg, menadione 10 mg, pyridoxine-HCl 15 mg, vitamin B_{12} 0.04 mg, vitamin D_3 20 mg (500 000 I.U. in 1 g), vitamin A (500 000 I.U. in 1 g) 30 mg, riboflavin 25 mg, BHT 50 mg. The vitamins were from Hoffmann La Roche, Basel, Switzerland.

Three subsamples were put into a cold extraction solution (5% trichloroacetic acid in 250 mM $HClO_4$ containing 0.08% EDTA), the next three were taken for dry matter determination (24 h at 85°C) and the rest immediately frozen for further analysis.

The samples in extraction solution were homogenized in an Ultraturrax (20–30 s) and centrifuged at 4°C 15000 × *g*, 30 min. Ascorbic acid and ascorbic sulfate were analyzed immediately or in thawed supernatants according to the method described by Carr et al. (1983) and Terada et al. (1978) as modified by Dabrowski and Hinterleitner (1989). The modification includes the background level determination of the interfering substances which form the color complexes with dinitrophenylhydrazine (DNPH). For this purpose part of the extract is placed directly in a water bath at 90°C and part is first kept with 1% $KBrO_3$ (Terada et al. 1978) to hydrolyze the ascorbic sulfate; both parts are then kept in the water bath for 1.5 h. This procedure destroys the ascorbic acid but in several cases (intestinal content) also releases sugars which form complexes with DNPH. Their maximum absorbance being at 440 nm. To estimate this possible interference for all types of samples the spectral absorbance from 600 to 400 nm is recorded with a Beckman DU-6 spectrophotometer and the difference in absorbance at 524 nm measured directly on scans.

The samples of food, faeces or alimentary tract content were dried at 85°C prior to metal analysis. In order to determine Cr, Zn, Fe and Cu, digestion of the samples was carried out in a 50 mL Kjehldal flask at 500°C for 15 h. After cooling 0.5 mL

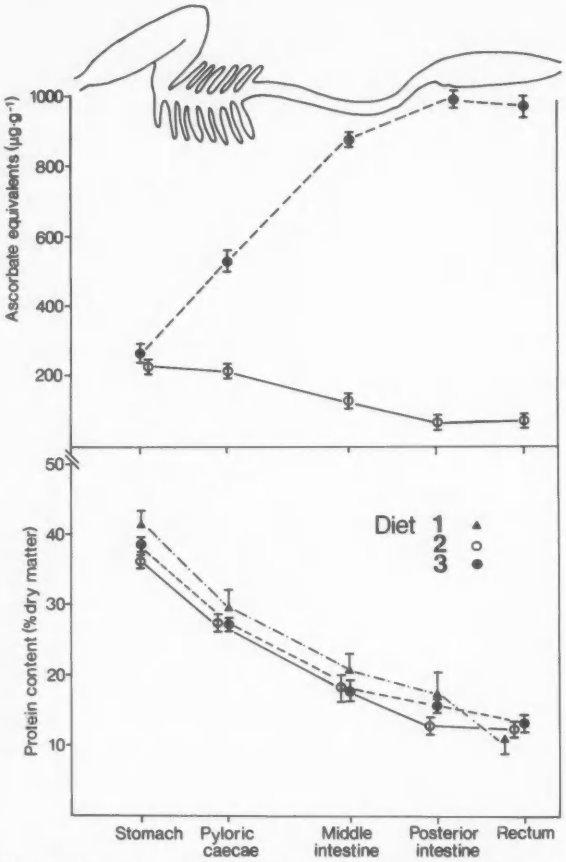

FIG. 1. Mean concentrations of AS and AA (*upper part*) and protein (*lower part*) in the digestive tract of rainbow trout. Vertical bars ± SD.

of an oxidation mixture containing 70% perchloric acid, concentrated sulphuric acid and 2% Na-molybdate were added to each ashed sample and heated until the colour changed from green to orange. After cooling 0.2 mL of perchloric acid was added and the sample heated again to boiling.

The digested samples were diluted with distilled water to the appropriate volume and metals measured by flame atomic spectrophotometry on a Perkin Elmer 2380 apparatus.

Lobster hepatopancreas was used as a standard for metal analysis (National Research Council Canada, Chemistry Division, Ottawa, Canada). Processing five samples of the standard by the dry digestion method described above resulted in Zn, Fe, and Cu 174 ± 12, 203 ± 29 and 372 ± 26 $\mu g \cdot g^{-1}$ dry matter, whereas according to the standard procedure the values should be 177 ± 10, 186 ± 11 and 439 ± 22. No correction was introduced in the results obtained in the present study.

Protein was analyzed in food and faeces according to the method of Lowry et al. (1957), with some modifications. The food sample (25 mg) was predigested in 1 *N* NaOH (10 mL) overnight, centrifuged and the solution used to construct the standard curve, since the protein content of the food was known.

For a casein–gelatin diet the linear part of the curve (50–300 µg protein) was described by the equation $P = 474.7(+5.15)A - 1.848$ where P is the amount of protein (µg) and A, the absorbance at 540 nm.

Apparent absorption of ascorbic acid and protein was calculated according to the formula given by Tacon and Rodriguez (1984) using Cr in food and faeces as the inert marker.

Data are mean values and standard deviation (*SD*) is given throughout. Statistical significance of differences in means was determined using the Student's *t*-test.

TABLE 2. Protein, mineral and marker content of the experimental diets. Mean ± SD. *Significantly different ($P<0.05$) in comparison to diet 1.

	Diet number		
Component	1	2	3
Protein (%)	51.03	53.34	53.13
Chromic oxide ($mg \cdot g^{-1}$)	6.06 ± 0.19	6.04 ± 0.44	5.22 ± 1.46
Zn ($\mu g \cdot g^{-1}$)	77.6 ± 7.3	94.6 ± 1.5*	93.6 ± 1.1*
Cu ($\mu g \cdot g^{-1}$)	12 ± 3	11 ± 0.8	15 ± 0.9
Fe ($\mu g \cdot g^{-1}$)	119 ± 4	137 ± 4*	159 ± 7*

Results

Both preparations, ascorbic acid (AA) and ascorbic sulfate (AS), were analyzed for purity by the DNPH method and by ultra violet spectral absorbance. The latter analysis revealed the maximum absorbance of 100 µM solutions in Na-acetate buffer pH 4.0 at 265 nm, to be 1.655 and 0.995 of AA and AS, respectively. Due to poor stability during processing and storage of vitamin C, we determined the recovery rate by analyzing the ascorbate content of freeze-dried pellets. Diet 2 contained 337.6 $\mu \cdot g^{-1}$ of total ascorbic acid of which 224.8 $\mu \cdot g^{-1}$ was in the form of dehydroascorbic acid. Diet 3 contained 357.3 $\mu g \cdot g^{-1}$ of ascorbic acid in the form of ascorbic sulfate, exclusively. Protein content and minerals varied between the diets (Table 2).

During the 28 d the fish increased in weight by 14.01 ± 3.4, 26.3 ± 5.0 and 17.6 ± 4.6% when fed diets 1,2 and 3, respectively, but the differences were not statistically significant. Fish fed diet 2 were characterized by a far better appetite than other groups, but to equalize food intake the feeding rate was adjusted to that of the group fed diet 1. However, the growth data must be interpreted with caution since only single experimental groups of ten fish were used and the mean final weights of fish were not significantly ($P<0.05$) different.

Dehydroascorbate (DHA) and ascorbic sulfate (AS) were found in faeces collected after three sub-periods (Table 3). Ascorbic acid was not found in the faeces of fish fed the diet depleted of this vitamin. DHA decreased significantly as compared to the amount in the food whereas the concentration factor (faeces/food ratio) for AS varied from 3.1 to 4.17. At the same time the marker added to the diet, chromic oxide, was concentrated 1.51–2.2 times. This suggests that speed of "flow" of the two substances through the alimentary tract of rainbow trout was significantly different.

Further evidence for faecal removal of AS in rainbow trout is that the stomach content of AS was lower than in food, but the intestinal content of AS increased gradually. DHA was the only form noted in the gut contents of the fish fed on diet 2 (Fig. 1) and amounted to 67.9 ± 4.2 $\mu g \cdot g^{-1}$ as compared with 337.6 $\mu g \cdot g^{-1}$ in the food. No traces of ascorbic acid were found in the gut content of fish fed diets 1 and 3.

In contrast to the ascorbic acid concentration in the gut contents of the three experimental groups of fish, the protein con-

TABLE 3. Dry matter (%), dehydroascorbic acid (DHA) or ascorbic acid sulfate (AS) ($\mu g \cdot g^{-1}$ dry weight), chromic oxide ($mg \cdot g^{-1}$ dry weight) and protein (% dry matter) in faeces of rainbow trout. N.D. — not detected. Mean ± SD

No. days after start of experiment		Treatment		
		Diet 1	Diet 2	Diet 3
7 d	Dry matter	963 ± 0.2	10.45 ± 0.05	9.64 ± 0.10
	DHA or AS	N.D.	113.2 ± 22.4	1104.4 ± 39.6
	Chromic oxide	11.13 ± 0.79	9.12 ± 0.84	10.33 ± 0.91
	Protein	11.89 ± 0.99	9.86 ± 0.7	9.92 ± 0.46
14 d	Dry matter	9.2 ± 0.7	10.1 ± 0.3	10.1 ± 0.1
	DHA or AS	N.D.	171.4 ± 30.5	1490.8 ± 100.4
	Chromic oxide	11.1 ± 0.65	11.2 ± 0.88	11.65 ± 1.6
	Protein	10.57 ± 0.28	10.39 ± 0.62	10.27 ± 0.71
21 d	Dry matter	12.0 ± 0.1	11.2 ± 0.1	11.6 ± 0.2
	DHA or AS	N.D.	130.2 ± 19.9	1136.1 ± 11.4
	Chromic oxide	9.91 ± 0.95	10.46 ± 1.4	8.20 ± 0.48
	Protein	11.05 ± 1.31	10.41 ± 1.47	10.41 ± 0.93

TABLE 4. Apparent absorption (%) of ascorbic acid (AA) and protein (P) in rainbow trout. Mean ± SD.

No. days after start of experiment		Treatment		
		Diet 1	Diet 2	Diet 3
7 d	AA	N.D.	77.9 ± 8.1	N.D.
	P	87.6 ± 6.2	87.8 ± 8.0	90.6 ± 7.8
14 d	AA	N.D.	72.7 ± 6.5	N.D.
	P	88.9 ± 5.2	89.6 ± 7.0	91.5 ± 12.5
21 d	AA	N.D.	77.8 ± 12.0	N.D.
	P	86.9 ± 8.3	88.7 ± 11.8	87.7 ± 5.1
28 d				
Gut segments[a]	P			
1		N.D.	21.9 ± 5.4	29.0 ± 6.5
2		46.4 ± 7.1	57.2 ± 5.5	52.7 ± 6.7
3		59.7 ± 11.2	77.2 ± 5.6	80.3 ± 2.1
4		76.8 ± 8.6	84.6 ± 10.9	82.5 ± 10.3
5		87.8 ± 9.3	86.9 ± 8.9	85.8 ± 15.0
	AA or AS			
1		N.D.	20.7 ± 5.1	27.7 ± 6.21
2		N.D.	44.12 ± 4.2	N.D.
3		N.D.	66.0 ± 4.8	N.D.
4		N.D.	87.2 ± 11.2	N.D.
5		N.D.	86.1 ± 8.8	N.D.

[a]Gut segments: 1 — stomach, 2 — pyloric caeca, 3 — middle intestine, 4 — posterior intestine, 5 — rectum (see also Fig 1. for details). N.D. — not detected.

TABLE 5. Mineral content in faeces of rainbow trout during the trial ($\mu g \cdot g^{-1}$ dry matter). Mean ± SD.

Sampling time		Diet number		
		1	2	3
7 d	Zn	302 ± 76	287 ± 48	253 ± 58
	Cu	46 ± 4	36 ± 2	55 ± 7
	Fe	516 ± 19	457 ± 100	527 ± 30
14 d	Zn	555 ± 51	409 ± 62	511 ± 70
	Cu	39 ± 7	32 ± 4	40 ± 5
	Fe	569 ± 96	676 ± 96	392 ± 68
21 d	Zn	198 ± 32	193 ± 75	185 ± 12
	Cu	36 ± 3	27 ± 1	37 ± 4
	Fe	304 ± 5	303 ± 32	289 ± 30

tent decreased gradually throughout the gut; there were no significant differences between treatments (Fig. 1).

The ascorbic acid concentration in food did not influence apparent protein absorption in the present trial (Table 4). Present data suggest that only 23.9 ± 2.4% of ascorbic acid is excreted in the faeces by rainbow trout, whereas perhaps the major pathway of ascorbic sulfate elimination after oral administration is by faecal excretion.

The present study suggests that some uptake of AS might take place in the fish stomach, but this must be clarified by further studies. The entire stomach content at the time of sampling, 16 h after the last feeding, was still several times larger than that of the remainder of the digestive tract. This may suggest that the "soluble" part of food containing AS was selectively released from the stomach faster than the less "soluble" part containing chromic oxide (data not shown). It seems that although a small proportion of AS can be absorbed in rainbow trout gut this is much less than is revealed by measurements of the apparent absorption coefficient. The present finding would suggest AA absorption rates in the stomach to be 20.7%, in the pyloric caecae region 23.4%, in the middle intestine 21.9%, and in the posterior intestine 20.1%.

The analysis of mineral present in the faeces (Table 5) revealed that although there was a very small variation of Cr content (Table 3) the other metals behaved differently. In most cases the mineral contents in faeces were the same whatever the dietary treatment, although great differences in concentration factors (faeces/food levels) were found between the three sampling times. The ratio of faeces to dietary contents were 1.9:–:7.1 for Zn, 1.8:–:4.9 for Fe and 2.4–3.7 for Cu. This rough estimate, when compared to Cr (concentration factor 1.5:–:1.8, see Table 3), would suggest that, based on faecal analysis, none of the minerals was absorbed to any great extent by rainbow trout fed on the experimental diets.

In the rainbow trout there were significant differences ($P<0.01$) in the Zn concentrations in the digestive tract contents (Fig. 2), and it seems that various portions of the gut are involved in the absorption and possibly secretion of this mineral. The trends and the differences in the Cu concentrations in

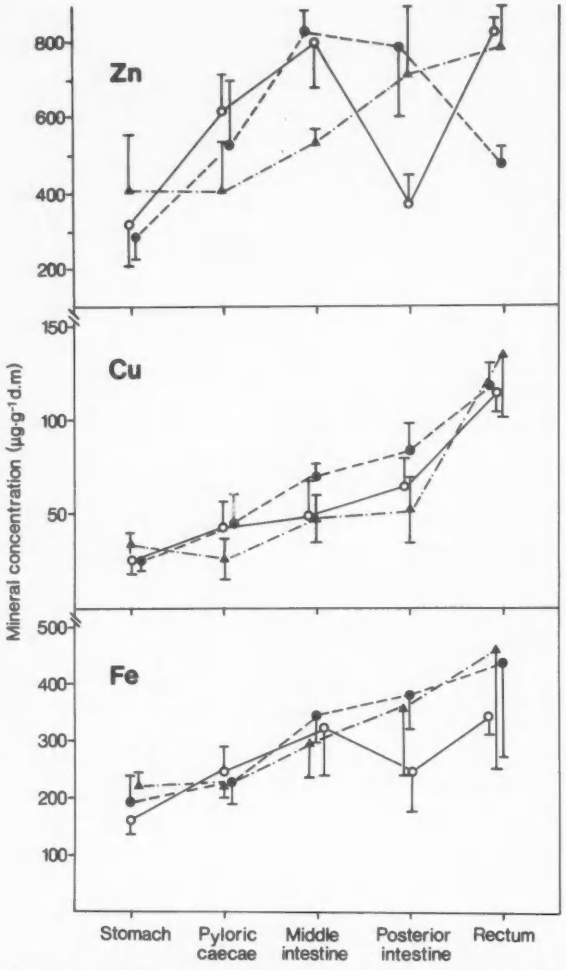

FIG. 2. Concentration of Zn, Cu, and Fe in the digestive tract contents of rainbow trout. Mean and ± SD (vertical bar) are shown.

the gut contents of the fish receiving the three experimental diets were not significant. It appears that higher absorption of Fe, corresponding to that of Zn, may take place in fish fed AA supplemented diet compared to the two other groups (Fig. 2).

Discussion

AS administered either orally or parenterally failed to prevent the onset of vitamin C deficiency symptoms in guinea pig (Kuenzig et al. 1974; Tsujimura et al. 1976) and monkey (Machlin et al. 1976). Tsujimura et al. (1982a) concluded that AS orally administered to humans was most probably excreted in the faeces since only 2.1–2.5% of the dose was recovered in the urine. Terada et al. (1978) found that when AS was given intraperitoneally or intravenously to rats the excretion in urine was several fold higher than after oral administration. Surprisingly, the suggestion made by Hornig (1975) and others, that in mammals AS is poorly absorbed and is excreted in the faeces, has not been followed up experimentally. The present results indicate that fish might not differ from higher vertebrates in the mechanism by which AS is handled in the alimentary tract. Negligible absorption of AS in fish raises doubts as to the value of this compound in fish diets.

The evidence of Tucker and Halver (1984, 1986) is based on (1) radioautographs showing that ingested labelled ascorbic sulfate is deposited in fish tissues, (2) prevention of scurvy in trout and catfish, (3) the presence of ascorbic sulfate in fish tissues as a natural storage form of vitamin C, and (4) the presence of ascorbate sulfate sulfohydrolase in trout liver.

Tsujimura et al. (1981) found some deposition of AS in the liver of four fish species, but the oral dose administered was over 200 times higher (762 $mg \cdot kg^{-1} \cdot d^{-1}$) than in the present study (3.05 $mg \cdot kg^{-1} \cdot d^{-1}$). When Tsujimura et al. (1982a) administered similarly high doses by oral tube for 4 d to coho salmon (*Oncorhynchus kisutch*) and ayu (*Plecoglossus altivelis*) they claimed to find an increase of total ascorbic acid in its tissues, suggesting that these fish were able to hydrolyze AS and utilize it as a source of vitamin C. Although differences between fish species is a plausible explanation, further reexamination of these results is required. Tsujimura et al. (1978) tested the AS antiscorbutic activity in rainbow trout juveniles in a feeding trial lasting 4–5 mo, whereas their experiment on guinea pigs lasted 2–3 wk (Tsujimura et al. 1976). In the former investigation AS activity as vitamin C was demonstrated by the lack of deficiency symptoms, the higher ascorbic acid level in the liver and the higher hydroxproline level in bones of fish fed a diet containing 1760 mg $AS \cdot kg^{-1}$, as compared to the corresponding levels in fish fed a diet depleted of vitamin C. Shapiro and Poon (1975) have already indicated that the stored AS is transformed to some other derivates, distinguishable by chromatographic methods from the original AS. The hydrolysis of AS when incorporated into food can be much accelerated and contribute to its utilization in the prolonged experiments on fish. We found that after 5 mo storage of crystaline form of AS at −20°C, 2% were hydrolyzed (present study). This could mean that in the experiment of Tsujimura et al. (1978) approximately 35 mg $AA \cdot kg^{-1}$ diet would be avilable within a few months after food preparation.

We demonstrated, the presence of dietary AA in the intestinal contents as DHA and suggest the absorption of this electroneutral compound in the gut brush border membranes in a Na-independent process (Bianchi et al. 1986). The antiscorbutic activity of DHA is well documented although the effectiveness of the substance in curing scurvy in the guinea pig is much lower than that of the reduced form, AA (Otsuka et al. 1986). It is therefore likely that dietary DHA has a lower bioavailability in ascorbic acid deficient animals.

Ascorbic acid is known to depress the absorption of copper, stimulate the absorption of iron and to have no effect on Zn absorption in guinea pig (Hornig et al. 1984). The promoting effect of AA, which acts as a reducing agent for ferric ion at acid pH, may enhance Fe-absorption several fold; this results in a 50–100% increase in the hepatic Fe-level (Milne and Omaye 1980; Smith and Bidlack 1980). A scorbutic diet induces a decrease in hepatic Fe and an increase in hepatic Cu.

In the present trial minerals were in a readily available form. In such cases an iron concentration of 265 $\mu g \cdot g^{-1}$ may lead to food refusal, increased mortality, less weight gain, and diarrhoea as manifested by a high level of faecal excretion in rainbow trout (Desjardins et al. 1987). In our experiments the dry matter of the intestinal contents did not differ significantly (data not shown), suggesting that the Fe dietary level, although close to excess, had no detrimental effect. Therefore, besides dem-

onstrating the higher Fe absorption in the posterior intestine of trout fed an AA diet (Fig. 2) the present study indicates that at Fe-levels over 120 $\mu \cdot g^{-1}$ ascorbic acid deficiency is unlikely to exacerbate iron deficiency symptoms.

Ascorbic acid supplementation had no effect on Zn and Cu concentrations of the kidney or liver of rainbow trout (Lanno et al 1985). In the present work, Zn levels in the gut contents of rainbow trout were variable, but showed no conclusive pattern despite the reduced levels of Zn in the rectum of the fish fed an AS diet. In other studies, Dabrowski et al. (1986) have found that Mg and other ions were reabsorbed in the posterior intestine of rainbow trout; this process was particularly pronounced in seawater fish. In rainbow trout fed diets based on commercial ingredients (fish meal, wheat meal, etc.) the ratios of dietary to faeces contents of Zn and Cu were much lower than that of Cr, suggesting some degree of absorption of these elements (Dabrowski et al. 1989).

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Size-Dependent Overwinter Mortality of Young-of-the-Year Yellow Perch (*Perca flavescens*): Laboratory, In Situ Enclosure, and Field Experiments[1]

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In laboratory experiments, fed and starved young-of-the-year yellow perch (*Perca flavescens*) lost weight when kept under simulated overwinter temperature and photoperiod regimes, small fish losing a greater proportion of their mass than larger ones. Perch in fed and starved treatments suffered 1 and 46% mortality, respectively, mortality being higher among the smaller individuals. Winter duration was an important determinant of both total mortality and the intensity of size-selective mortality. Size-selective mortality also occurred in yellow perch from the same stock kept overwinter in an in situ lake enclosure with natural food. Fall and spring population estimates for two cohorts of young-of-the-year yellow perch from Lake St. George also indicated the occurence of size-selective overwinter mortality. We present a quantitative technique for identification of size-dependent mortality and size-dependent growth from sequential length frequency distributions. This technique allowed identification of overwinter size-selective mortality for five natural cohorts sampled in the field. Results from a stochastic simulation model, incorporating observed variability in both first year growth and winter duration suggest that overwinter starvation mortality can cause substantial variability in year-class strength that is independent of adult stock size.

Au cours d'expériences en laboratoire, des jeunes perchaudes (*Perca flavescens*) de l'année nourries ou privées de nourriture exposées à des conditions hivernales simulées de température et de photopériode ont vu leur poids diminuer. Les plus petits poissons ont perdu une plus grande proportion de leur poids par rapport aux poissons de plus grande taille. Les taux de mortalité des perchaudes nourries ou privées de nourriture étaient respectivement de 1 % et de 46 %, la mortalité étant supérieure parmi les individus de plus petite taille. La durée du maintien des conditions hivernales était un facteur important en ce qui a trait à la mortalité totale et à l'intensité de la mortalité sélective selon la taille. Nous avons également observé une mortalité sélective selon la taille chez des perchaudes du même stock gardées durant l'hiver dans un enclos lacustre naturel dans lequel elles trouvaient leur nourriture. En outre, les estimations, réalisées à l'automne et au printemps, des populations de deux cohortes de jeunes perchaudes de l'année du lac St-George ont également révélé l'existence d'une mortalité sélective selon la taille au cours de l'hiver. Nous présentons une méthode quantitative permettant d'établir, à partir des distributions séquentielles des fréquences de longueur, la mortalité reliée à la taille et le taux de croissance en fonction de la taille des individus. Cette méthode a permis d'établir la mortalité hivernale liée à la taille dans le cas de cinq cohortes naturelles échantillonnées sur le terrain. Les résultats obtenus à partir d'un modèle stochastique de simulation, tenant compte de la variabilité observée dans la croissance des individus durant la première année de même que de la variabilité dans la durée de l'hiver, laissent penser que la mortalité hivernale due à la privation de nourriture peut entraîner une variabilité importante dans les effectifs des différentes classes d'âge, variabilité indépendante de la taille du stock de poissons adultes.

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Annual variation in recruitment of yellow perch, *Perca flavescens*, and Eurasian perch, *P. fluviatilis*, is typically high (Nielsen 1980; Craig and Kipling 1983; Henderson 1985). Several factors have been identified as possible regulators of year-class strength in perch including stock size (Craig 1982; Craig and Kipling 1983; Evans and Waring 1987), temperature (Craig 1982; Craig and Kipling 1983), ice cover (Kipling 1976), water levels (Henderson 1985), and interspecific interactions (Tarby 1974; Nielsen 1980; Craig and Kipling 1983; Post and Prankevicius 1987; Post and Evans 1989). One feature which might be common to all these factors is size-dependent survival. For example, growth rate of young-of-the-

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year (YOY) yellow perch is, in many cases, positively correlated with water temperature (Koonce et al. 1977; Craig 1982), and predation mortality can be related negatively to size (Nielsen 1980; Post and Prankevicius 1987; Post and Evans 1989). Thus temperature can influence recruitment through effects on growth and body size. Similarily, density-dependent growth (Craig and Kipling 1983; Henderson 1985; Mills et al. 1987) and hence size-dependent mortality might influence recruitment, although there is no direct evidence that stock-recruitment relationships are modified by YOY body size.

Two size-linked mechanisms have been hypothesized to explain recruitment variability in perch populations: (1) size-dependent predation (Nielsen 1980; Craig 1982; Post and Prankevicius 1987), including cannibalism (Tarby 1974; Post and Evans 1989); and (2) overwinter mortality (Toneys and Coble 1980). The latter hypothesis is the subject of our study. The smaller individuals within a cohort have higher maintenance requirements per unit body mass than the larger ones (Paloheimo and Dickie 1966). During winter at northern latitudes when activity is slowed by cold temperatures and planktonic prey is at a low density, smaller individuals would be expected to exhaust stored energy earlier and subsequently be more subject to overwinter mortality due to starvation.

Field evidence of size-selective overwinter mortality in YOY fish is of two types. The first consists of comparisons between fall and spring length-frequency distributions examples of which are studies on bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*) by Toneys and Coble (1979) and on smallmouth bass (*Micropterus dolomieui*) by Shuter et al. (1980). This evidence is inconclusive if size-dependent growth occurs between the sampling periods. The second type of evidence is a postive correlation between body size and overwinter survival as has been presented for brook trout (*Salvelinus fontinalis*) by Hunt (1969). This correlative evidence suggests that cohorts of fish with larger mean body size suffer less overwinter mortality.

Laboratory evidence of size-selective overwinter mortality can be more conclusive because direct observation is possible, but in some cases the results contradict the patterns observed in the field. For instance, Toneys and Coble (1980) found no size-related mortality and very little total mortality in green sunfish (*Lepomis cyanellus*), largemouth bass, and brook trout kept overwinter in the laboratory at winter temperatures. However, they did observe size-related mortality in yellow perch. Similarly, Oliver et al. (1979) observed size-related mortality in overwintering YOY smallmouth bass in the laboratory.

Our aim is to evaluate the extent of overwinter mortality for YOY yellow perch. The specific objectives include: (1) a test of the hypothesis that size-selective overwinter mortality occurs under simulated winter conditions in the laboratory, (2) a quantification of overwinter mortality patterns with respect to body size and the duration of the winter period, (3) the development of a quantitative technique to separate effects of size-dependent overwinter mortality from those of size-dependent growth, and (4) an evaluation of overwinter mortality in near natural (in situ enclosure) and natural field conditions.

Materials and Methods

Laboratory Experiment

YOY yellow perch for the laboratory experiment were captured by beach seining in Dickie Lake, Ontario (Table 1), between October 30 and November 7, 1985. The fish were transported to the laboratory and kept in a 455 L holding tank at ambient Dickie Lake temperature until November 12, 1985. They were then sorted into 2 mm length categories. An equal number of fish were chosen at random from each length category for each of four experimental groups. Approximately 82 yellow perch were assigned to each experimental group. All fish were measured to the nearest millimetre, weighed to the nearest 0.05 g, and placed in insulated wooden tanks ($72 \times 42 \times 26$ cm, 79 L) which were coated with non-toxic grey paint and had glass fronts and styrofoam lids. A window in the laboratory provided natural light and a seasonal photoperiod regime corresponding to 44° N latitude. The tanks were supplied with a continuous flow (660 mL min^{-1}) of refrigerated well water. Temperatures in the tanks were maintained throughout the experiment at 4.0°C (SD = 0.02°C) by use of a single supply reservoir with a thermostatically controlled heater. Temperature was recorded automatically and continuously at the supply reservoir and checked manually once daily in each experimental tank.

TABLE 1. Study lake location and morphometry.

Lake	Location (Long., Lat.)	Area (ha)	Depth (m) Mean	Depth (m) Maximum
St. George	34°57′N, 79°25′W	10.4	5.7	15.0
Harp	45°23′N, 79°08′W	66.9	12.4	40.0
Dickie	45°09′N, 79°05′W	93.2	5.0	12.0
Clear	45°12′N, 78°43′W	96.0	12.4	33.0

Duplicates were run for each of the starved and fed treatments. The fish in the starved treatment remained unfed for the duration of the experiment and the fish in the fed treatment were fed once daily, ad libitum, with a combination of live zooplankton, freeze dried zooplankton and euphausids, and frozen brine shrimp. After feeding, uneaten food always remained on the bottom of the tanks. Feces and uneaten food were siphoned from the tanks, approximately weekly. Dead fish were removed daily and their total lengths recorded. The experiment was run for 174 d. Fish remaining alive at the end of the experiment were counted, measured, and weighed.

Field Enclosure Experiment

Yellow perch for the overwinter enclosure experiment were also from the Dickie Lake stock. They were measured and weighed using the same procedure as for the laboratory experiment. On December 6, 1985, 110 perch were stocked through the ice into an enclosure in Lake St. George (Table 1). The enclosure was 8 m in diameter, 15 m deep and constructed of polyvinyl-coated nylon which completely isolated a column of water from the surrounding lake. It was open to the water–sediment interface. Details of construction and maintenance are given in McQueen et al. (1986) and Post and McQueen (1987). Seasonal temperature and oxygen regimes and the zooplankton and phytoplankton communities of enclosures of this type are typical of the surrounding lake (McQueen et al. 1986; Post and McQueen 1987). On May 7–16, 1986, a fine-mesh (1 X 1 mm) purse seine (30 m long, 9 m deep) similar in design to that described by Evans and Johannes (1988) and baited minnow traps were fished repeatedly to remove surviving yellow perch from the enclosure. Captured yellow perch were measured for total length and weighed to the nearest 0.05 g.

Field Studies

YOY yellow perch from four lakes (Table 1) were sampled in the fall and subsequent spring. Two approaches were taken in Lake St. George. First, population abundance estimates were made during the fall and following spring for each of two year-classes to estimate overwinter mortality. Population sizes were estimated using a multiple census mark–recapture method (Schnabel 1938) from August 27 to September 25, 1984 and May 27 to June 11, 1985 for the 1984 year-class, and from August 21 to September 18, 1985 and May 28 to June 11, 1986 for the 1985 cohort. Perch were captured in the littoral zone with a fine mesh (1 X 1 mm) beach seine (30 m long X 1.5 m deep), and in the pelagic zone with the same purse seine used in the enclosure. In each experiment, approximately 5–10% of the population was marked by clipping approximately 2 mm of either the upper or lower tip of the caudal fin. Clipped perch suffered no mortality during several 3 mo long enclosure experiments during the summer and fall, nor in tanks with flow-through lake water maintained throughout each mark–recapture experiment. It was therefore assumed that the handling and clipping of the fish caused no mortality. The second approach taken in Lake St. George was to compare fall and spring length-frequency distributions as was done for the laboratory and enclosure data. Sampling was conducted from October 2 to 3, 1984 and April 23 to 24, 1985 for the 1984 year-class and from October 19 to 24, 1985 and April 15 to 17, 1986 for the 1985 year-class.

Fall and spring length-frequency distributions were also observed in three other study lakes (Harp Lake: September 13, 1985 and May 6, 1986; Dickie Lake: September 12, 1985 and May 5, 1986; Clear Lake: September 14, 1985 and May 14, 1986). For these lakes, length-frequency distributions were determined from catches caught with the same gear as was used for the Lake St. George mark–recapture experiments.

Estimates of winter duration were made from mid-lake temperature profiles taken in the study lakes at weekly to monthly intervals from 1981 to 1986 in Lake St. George (D. J. McQueen, York University, Toronto, unpublished data), from 1976 to 1986 in each of Harp and Dickie Lakes (Reid et al. 1983, Reid and Girard 1985, R. A. Reid, Ontario Ministry of the Environment, Dorset, Ontario, unpublished data) and from 1985 to 1986 in Clear Lake (R. A. Reid, unpublished data). Winter duration was defined as the number of days that the mean epilimnetic water temperature was ≤6°C. This temperature corresponded approximately to that at which the swimming and feeding activity of YOY yellow perch in the laboratory was reduced.

Results

Laboratory Experiment

The size distributions of YOY yellow perch did not differ among the four experimental tanks at the start of the experiment (Chi-square, $\chi^2 = 23.58$, df = 30, $P > 0.05$), or between duplicates at the end of the experiment (Chi-square, starved treatment: $\chi^2 = 5.68$, df = 10, $P > 0.05$; fed treatment: $\chi^2 = 4.38$, df = 10, $P > 0.05$). We therefore combined the duplicates within treatments for all subsequent length-frequency analyses.

Length–weight regressions for fish in the four tanks in the fall had equivalent slopes (ANCOVA, $F = 1.14$, $P > 0.05$) and intercepts (ANCOVA, $F = 2.05$, $P > 0.05$). Length–weight regressions for the duplicates in the fed treatment in the spring had equivalent slopes (ANCOVA, $F = 0.28$, $P > 0.05$) and intercepts (ANCOVA, $F = 0.85$, $P > 0.05$). Length-weight regressions for the duplicates in the starved treatment in the spring had equivalent slopes (ANCOVA, $F = 0.27$, $p > 0.05$) and intercepts (ANCOVA, $F = 2.34$, $P > 0.05$). We therefore combined the duplicates within treatments for all subsequent length–weight analyses:

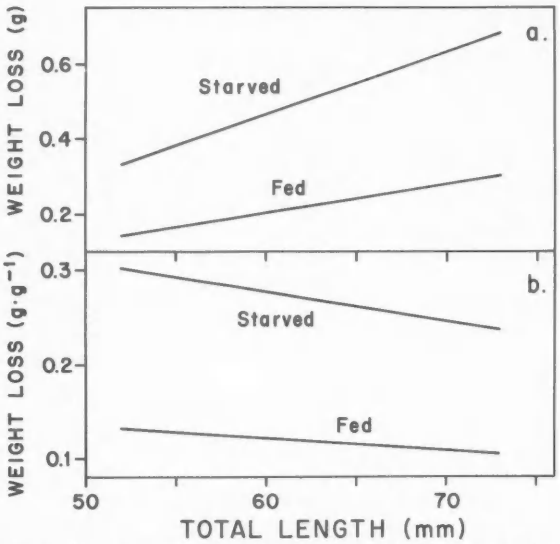

FIG. 1. Overwinter size-dependent loss of weight by young-of-the-year yellow perch in the starved and fed laboratory treatments expressed as (a) absolute loss and (b) as a proportion of initial body weight. Since individuals in the experiment were not marked, the curves were derived from the difference between fall and spring length to weight relationships.

Fall	$WT = 1.02 \times 10^{-5} TL^{2.92}$ ($n = 329$, $r^2 = 0.94$)
Spring-Fed	$WT = 6.72 \times 10^{-6} TL^{2.99}$ ($n = 164$, $r^2 = 0.80$)
Spring-Starved	$WT = 2.65 \times 10^{-6} TL^{3.17}$ ($n = 134$, $r^2 = 0.88$)

where WT is wet weight in g and TL is total length in mm.

Weight loss occurred in both the starved and fed treatments over the course of the experiment. The slope of the length versus weight regression was significantly lower in the fall than in both the fed and starved treatment in the spring (ANCOVA, $F = 220.40$, $P < 0.05$; $F = 1989.87$, $P < 0.05$, respectively). Therefore, there was a significant positive relationship between weight loss and fish total length (Fig. 1a), smaller fish losing relatively more weight than larger ones. There was no difference in slope between the two treatments in the spring (ANCOVA, $F = 1.23$, $P > 0.05$), but the intercept was lower in the starved treatment (ANCOVA, $F = 261.89$, $P < 0.05$). This indicated that the starved fish lost significantly more weight than did the fed fish (Fig. 1a). When weight loss from fall to spring was expressed as a proportion of the fall weight, it was apparent that small fish lost proportionately more weight than did larger fish (Fig. 1b). In parallel with this, during the experimental period, starved fish suffered 46% mortality (77/164), fed fish only 1% mortality (1/165). Therefore, YOY perch, whether fed or not, lost weight in a size dependent manner, but only the starved ones lost sufficient weight to suffer significant mortality.

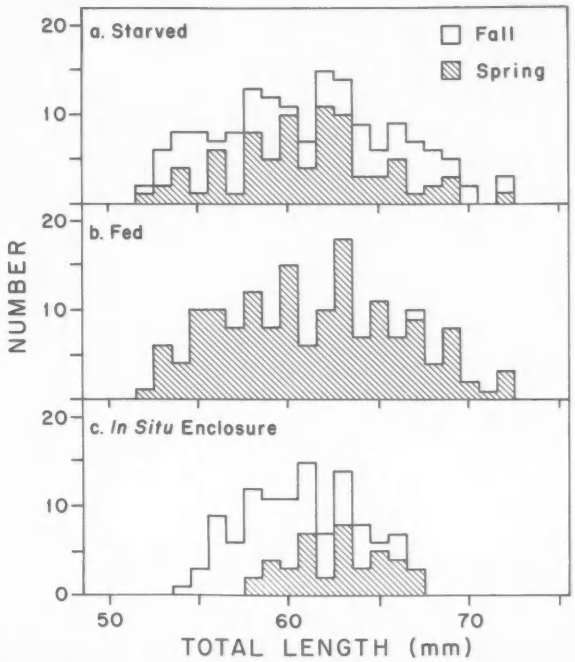

FIG. 2. Fall and spring length-frequency distributions of young-of-the-year yellow perch in the laboratory (a) starved and (b) fed treatments and in the (c) in situ enclosure.

The initial and final length-frequency distributions of the starved (Fig. 2a) and fed treatment (Fig. 2b) did not differ (Chi-square, $\chi^2 = 6.51$, df = 10, $P > 0.05$ and $\chi^2 = 0.06$, df = 10, $P > 0.05$, respectively), but the temporal pattern of mortality among starved fish was size-dependent. Only small individuals died during the initial 100 d of the experiment, but as winter duration increased, larger YOY also died. To quantify this relationship, the probability of mortality on a particular date was calculated for each of eleven, 2 mm size classes from 52–53 to 72–73 mm TL, by dividing the number that had died by a particular date by the total number of perch in that size class at the beginning of the experiment. This is the same method used by Shuter et al. (1980) in their analysis of the laboratory overwinter mortality data collected by Oliver et al. (1979). The resulting data set is a matrix of simulated daily winter durations, with calculated probabilities of mortality for each day for each of the length classes. A multiple regression model was then used to determine the relationship between the probability of mortality, perch total length and simulated winter duration:

$$\text{PMORT} = 16.71 * e^{0.02554\ \text{WD} - 0.1381\ \text{TL}} \qquad (R^2 = 0.66,\ n = 487)$$

where PMORT is the probability of mortality, WD is the simulated winter duration in days and TL is perch total length in mm. The coefficients for both independent variables were significant (*t*-test, WD: $t = 23.88$, $P < 0.05$; TL: $t = -25.51$, $P < 0.05$). Therefore, the combination of fish size and winter duration is necessary to adequately describe the pattern of mortality observed in the laboratory experiment (Fig. 3).

Field Enclosure Experiment

Fall and spring length frequency distributions of the Dickie Lake yellow perch in the in situ enclosure in Lake St. George

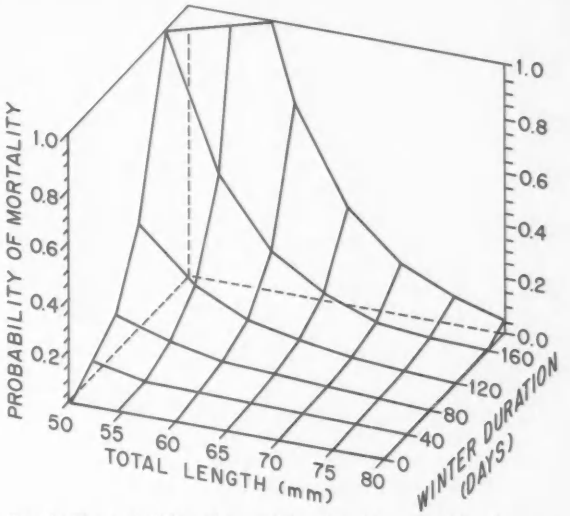

FIG. 3. The probability of overwinter starvation mortality of young-of-the-year yellow perch as related to fall total length and winter duration.

differed significantly (Chi-square, $\chi^2 = 10.26$, df = 4, $P < 0.05$). The distributions (Fig. 2c) suggested that both size-selective loss of smaller individuals and growth in length occurred between December 6, 1985 and May 7, 1986. The enclosure was predator-free and therefore the observed loss could not have been due to predation. It was possible, but unlikely, that survivors were missed in this sampling which would have caused an overestimate in the total mortality calculated from the difference in numbers stocked in the fall and recaptured in the spring. There was no reason, however, to suspect that this type of sampling error would have acted in a size-selective manner.

Separating Size-Selective Mortality From Growth Responses

To identify the effect of size-dependent mortality on a length-frequency distribution, we have employed empirical quantile–quantile (QQ) plots (Chambers et al. 1983). This method allows a comparison between two distributions by describing their shapes with quantiles. In a simulated normal distribution with a mean of 60 mm and a standard deviation of 2 mm, which is typical of fall distributions of YOY yellow perch in five southern Ontario lakes (Post 1987), quantiles can be used to describe the shape of the distribution (Fig. 4a). Quantiles 1, 5, 10, 25, 50, 75, 90, 95, 99 are distributed approximately evenly along the length axis and therefore will reduce a univariate frequency distribution to a linear relation, facilitating comparison with a second such transformed distribution. We then applied our laboratory derived overwinter size-dependent mortality function to this simulated normal distribution. Figure 4b shows the effect of our empirically derived mortality model on the normal length-frequency distribution and on the position of quantiles on the length axis. A visual comparison of the length-frequency distributions in Fig. 4a and 4b suggests little change in shape since they are still approximately symmetrical, with a slight increase in the mean. The distributions do not differ (Chi-square, $\chi^2 = 6.55$, df = 11, $P > 0.05$). This simulation, with a winter duration of 180 d, produced a total mortality of 43%,

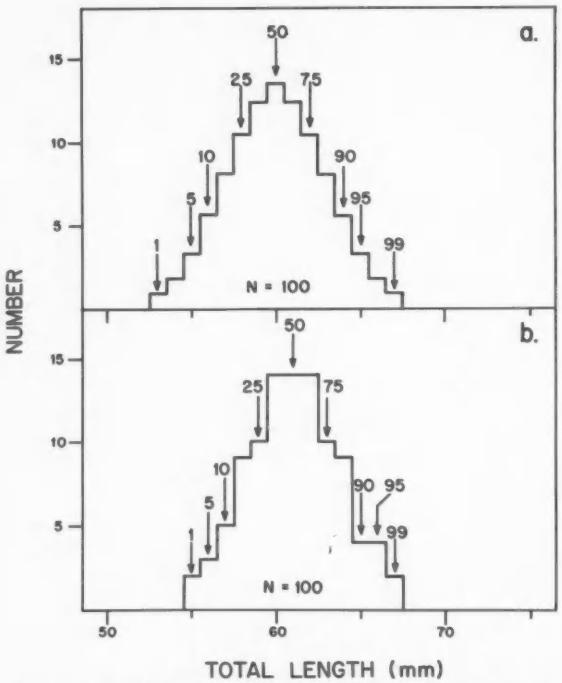

FIG. 4. Simulated length-frequency distributions (a) normal distribution with a mean of 60 mm and a standard deviation of 2 mm and (b) distribution predicted from application of the laboratory model to the normal distribution, with a winter duration of 180 d. Sample sizes and position of quantiles indicated.

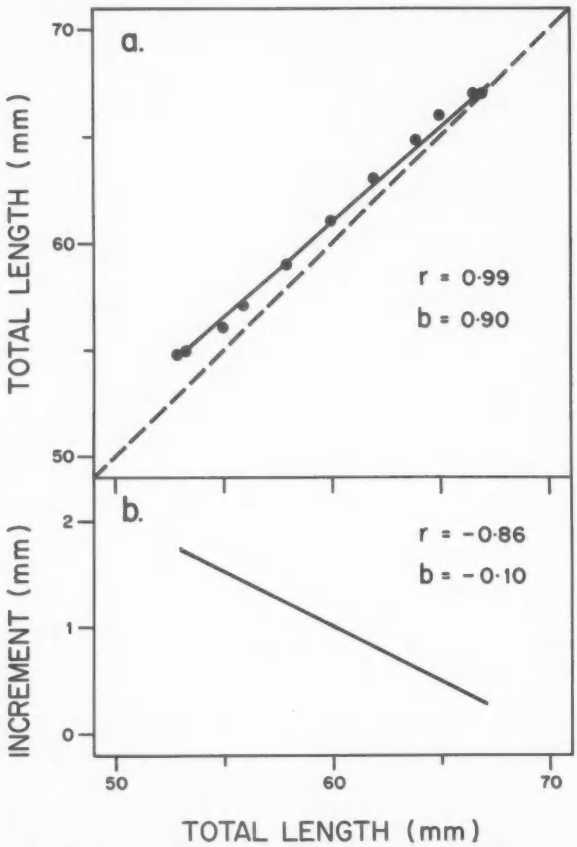

FIG. 5. (a) The empirical quantile–quantile plot derived from the distributions from Fig. 5a (x axis) and Fig. 5b (y axis) and (b) the incremental difference between the calculated empirical quantile–quantile regression and the 1:1 line. Correlation coefficients (r) and regression slopes (b) are indicated.

but the distributions, despite size-selective losses, did not differ statistically using the chi-square statistic. A QQ plot of the initial normal distribution on the x-axis and distribution resulting from size-selective mortality on the y-axis demonstrates how this technique can be used to identify changes in shapes of distributions due to size-selective mortality (Fig. 5a). The slope of the line is less than 1.0 as compared to the 1:1 line. A plot of the incremental difference between the normal and simulated quantiles has a negative slope (Fig. 5b). Both of these plots diagnose the effects of a size-dependent mortality function on a length-frequncy distribution, but the increment plot is more effective visually.

As suggested by the enclosure experiment, the occurrence of growth between the samplings of fall and spring length frequencies could interfere with the test for overwinter size-dependent mortality. In natural populations, YOY perch can not be reliably captured after fall turnover or before spring stratification. Therefore, some growth typically occurs between fall and spring sampling, most probably during late fall and early spring.

If there is no growth between sampling periods, interpretation of the quantile–quantile and increment plots is straightforward (Fig. 6, and 7, Table 2). Size-selective mortality of small individuals (Case 2) produces a slope < 1 on the QQ plot and negative slope on the increment plot with deviations from the 1:1 line being positive. Size-selective mortality of large individuals (Case 3) produces a QQ slope < 1 and a negative slope on the increment plot, but the deviations are negative. Under the scenario of no size-selective mortality and if the absolute incremental change in length is the same for all sizes (Case 4), then the length-frequency distribution will remain unchanged in shape and will shift to the right along the length axis. The response of the QQ plot will be an upward translation with the slope remaining 1. If the incremental change in length is less for smaller than larger individuals (Case 7), the QQ plot will be translated up, more for larger individuals, producing a counter-clockwise rotation and a slope > 1. The increment plot will have a positive increment and slope. Under this scenario, the length-frequency distribution will change in shape, spanning a greater length range.

Growth scenarios 4 and 7 have been noted for YOY fish in the wild. Largemouth bass 10–30 mm in length had daily growth rates that were independent of body size as in Case 4, whereas larger ones grew in a size-dependent manner, with larger fish growing faster as in Case 7 (DeAngelis and Huston 1987). Size-dependent growth of older YOY fish is the pattern most often observed in nature (DeAngelis and Coutant 1979; Keast and Eadie 1984; Wismer et al. 1985; DeAngelis and Huston 1987). The final growth scenario is that the increment is greater for smaller than larger individuals. This would result in a sequential decrease in length range through time. This growth scenario

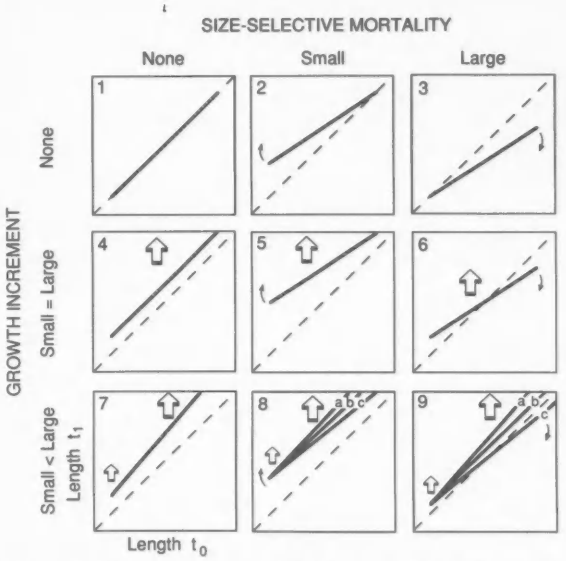

FIG. 6. Responses of empirical quantile–quantile plots to combinations of size-selective mortality of small and large individuals and to growth models in which the incremental change in length of small individuals is equal to or less than that of large individuals. The dashed line is the 1:1 line. The panel number refers to Case number in Table 2. The open arrows indicate the direction of the translation due to growth effects, the size of the arows indicating the relative magnitude of the response. The curved arrows indicate the direction of the rotation due to size-selective mortality.

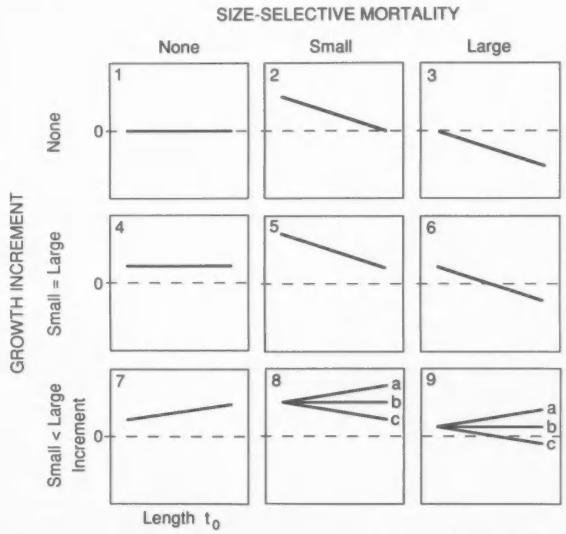

FIG. 7. Responses of increment plots to combinations of size-selective mortality of small and large individuals and to growth models in which incremental change in length of small individuals is equal to or less than that of large individuals. The panel number refers to Case number in Fig. 6 and Table 2.

will not be considered further as it is unlikely to occur in natural populations.

Potential combinations of size-selective mortality and growth shown in Fig. 6 and 7 and Table 2 indicate the possibility of similar QQ and increment plot slopes and signs for quite different mortality and growth scenarios. For example, case 7 and 8a are similar as are cases 4 and 8b, 5 and 8c, and 1 and 9b. Thus, while certain responses can not be seperated using the simple slope and position diagnostics of the QQ and increment plots, the various potential patterns of response are revealed by this approach and the difficulties of interpretation are clarified.

In situations where both size-dependent mortality and size-dependent growth occur (cases 8 and 9), the response of the QQ and increment plots will depend on the magnitude of the countervailing forces. In all cases where QQ slope $\neq 1$ and the increment slope $\neq 0$, a size-dependent mechanism has acted to change the shape of the distribution. The interpretation of these diagnostics will depend on specific information on the magnitude of the size-dependent processes occurring.

Application of this technique to results from the laboratory and enclosure suggests that it can be used to identify size-selective mortality using real, non-normal data. An increment plot for starved fish, which were observed to suffer size-selective mortality of small individuals, had a negative slope which was significantly different from zero (Fig. 8a) analogous to case 2. The same plot for the fed treatment, which did not suffer size-selective mortality, had a slope not significantly different than zero (Fig. 8a) analogous to case 1. These plots also suggested that there was some shrinkage in length, more so in the starved than fed fish, as evidenced by the negative increments. The increment plot for the fall and spring length distributions in the enclosure experiment had a positive increment and negative slope, significantly less than zero (Fig. 8c). Since growth between the late fall addition of the yoy perch to the enclosure and spring retrieval was minimal, the QQ analysis was also analogous to case 2.

The technique can, therefore, be used to identify size-selective mortality of the smaller individuals within a natural cohort from fall and spring length-frequency distributions observed in the field. If the size-related growth pattern is known, the magnitude of the increment plot can be used to quantify the relative intensity of the size-selective mortality of a cohort observed between years in a single population or between populations. A large negative slope indicates intense size-selective mortality of small individuals, a smaller negative slope indicates a more subtle size-selective mortality of small individuals.

Overwinter Mortality in Natural Populations

The 1984 yellow perch cohort in Lake St. George suffered 76% mortality between the fall of 1984 ($N = 119\,000$; 95% confidence intervals 104 000–136 000) and the spring of 1985 ($N = 29{,}000$; 95% confidence intervals 26 000–32 000), while the 1985 cohort suffered 42% mortality from the fall of 1985 ($N = 52\,000$; 95% confidence intervals 44 000–60 000) to the spring of 1986 ($N = 31\,000$; 95% confidence intervals 28 000–35 000). The 1984 cohort was relatively slow growing and had a mean length of 60.7 mm by October 1 (Fig. 9). In contrast, the 1985 cohort attained a mean length of 71.4 mm by October 20. Therefore, the cohort with smaller fish suffered 34% higher mortality than did the cohort with larger ones.

Mortality rates of the 1984 and 1985 yellow perch cohorts from Lake St. George were calculated from the decrease in abundance from fall to spring, based on the mark–recapture estimates. Since these estimates were conducted in early fall and mid-spring, the estimated mortality rates included that dur-

TABLE 2. Responses of empirical quantile–quantile plots and increment plots to various combinations of size-selective mortality of small and large individuals and growth models in which incremental change in length of small (S) fish is = or < that of large (L) fish.

Case	Process		Q–Q Plot Response	Increment plot	
	Growth increment	Differential mortality		Sign	Slope
1	None	None	None	0	0
2	None	Small	No translation; Clockwise rotation	+	−
3	None	Large	No translation; Clockwise rotation	−	−
4	S=L	None	Translation up; No rotation	+	0
5	S=L	Small	Translation up; Clockwise rotation	+	−
6	S=L	Large	Translation up; Clockwise rotation	+,−	−
7	S<L	None	Translation up; counter-clockwise rotation	+	+
8	S<L	Small	Translation up; Countervailing rotations	+	+,0,−
9	S<L	Large	Translation up; Countervailing rotations	+,0,−	+,0,−

ing late fall and early spring. Between mid-points of the fall and spring mark–recapture estimates, 286 and 294 d elapsed during 1984 and 1985 "overwinter" periods. The observed winter duration was 158 and 146 d for the 2 yr. Therefore, the mortality rates reported for the two Lake St. George cohorts were probably overestimates of the actual overwinter mortality.

Total overwinter mortality for the two Lake St. George cohorts and those from Harp, Dickie, and Clear lakes were estimated using the laboratory model, observed fall length-frequency distributions and observed winter durations. The estimated overwinter mortality of the 1984 year-class from Lake St. George was 24% compared to 4% for the 1985 year-class. There were two reasons for this difference. First, fish from the 1984 cohort were smaller, and second, the winter (WD) was longer in 1984–85 than in 1985–86 (158 versus 146 d). The estimated overwinter mortality of the 1985 cohorts from Harp, Dickie, and Clear lakes were 29, 36, and 57%, with winter durations of 144, 144, and 151 d, respectively.

The QQ analysis of the Lake St. George length-frequency data indicated a change in shape from the fall to spring (Fig. 10). The increment plots for both years had positive increments and significant negative slopes. These results were potentially consistent with two scenarios: (1) overwinter size-selective mortality of small individuals and low growth rates (cases 2, 5, or 8c) and (2) overwinter size-selective mortality of large individuals and high growth rates (cases 6 or 9c). The second scenario was unlikely given that the YOY perch in the in situ enclosure in Lake St. George during the winter of 1985–86 grew minimally. We therefore concluded that the two Lake St. George cohorts suffered size-dependent mortality favouring the survival of the larger members of the cohort. The negative slope of the increment plot was greater for the smaller fish of the 1984 cohort suggesting a more intense size-selective mortality. This was consistent with the size-dependent pattern in the laboratory experiment and the mark–recapture population estimates.

Overwinter shifts in length-frequency distributions of YOY yellow perch in Dickie, Harp, and Clear lakes all suggest the occurence of size-dependent processes (Fig. 9). The increments were all positive and the slopes were negative and significantly less than zero for Harp and Dickie lakes and negative but not significant for Clear Lake (Fig. 10). Growth between the fall and spring sampling periods in these lakes obscured the conclusion, but it was clear that growth alone (case 4 or 7) could not have accounted for the observed change. A combination of size-dependent mortality and size-dependent growth was necessary to explain the differences between the fall and spring length-frequency distributions. To maintain consistency between observations from experiments in which growth was minimal (fed and starved laboratory treatments, in situ enclosure and Lake St. George 1984 and 1984 cohorts) and growth patterns typical of YOY fish, we concluded that the YOY perch in Dickie, Harp, and Clear lakes suffered size-selective mortality of the smaller individuals producing QQ and increment responses analogous to case 8c.

Overwinter Mortality and Variability In Year-Class Strength

Annual variation in overwinter mortality within a population is expected to be a function of both variability in YOY growth rates and in winter duration. To estimate the expected magnitude of this variability within and between populations we developed a stochastic simulation model. The model incorporated variability in winter duration, variability in mean fall body size and a normal length-frequency distribution about the mean fall body size.

Simulations for each of our study lakes were run with observed lake specific mean and variability in winter duration (St. George: mean = 154.2 d, SD = 7.3 d, N = 6 yr; Harp: mean = 164.2 d, SD = 9.3 d, N = 10 yr; Dickie: mean = 163.4 d, SD = 8.6 d, N = 10 yr). Water temperature data from Clear Lake was insufficient to derive winter duration data for winters other than 1985–86. Clear lake is in the same general location as Harp and Dickie lakes (Table 1) but is more sheltered by the surrounding topography. This may explain why Clear Lake had a 7 d longer winter duration than Harp or Dickie lakes in the

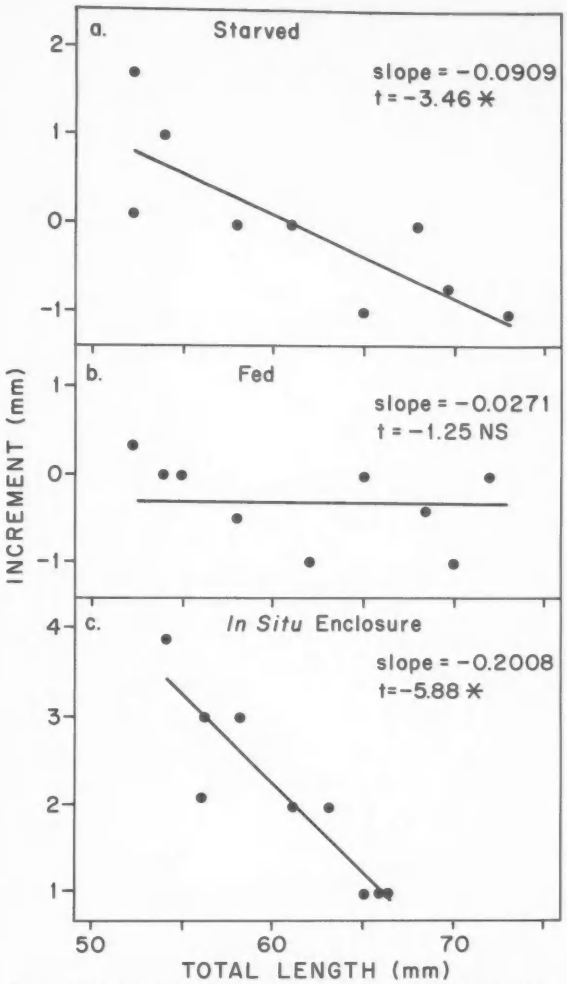

FIG. 8. Increment plots from the (a) starved and (b) fed treatments in the laboratory and the (c) in situ enclosure experiments. Slopes and *t*-test for the hypothesis that $b \geq 0$ are indicated. Significant ($P<0.05$) negative slopes are indicated by *.

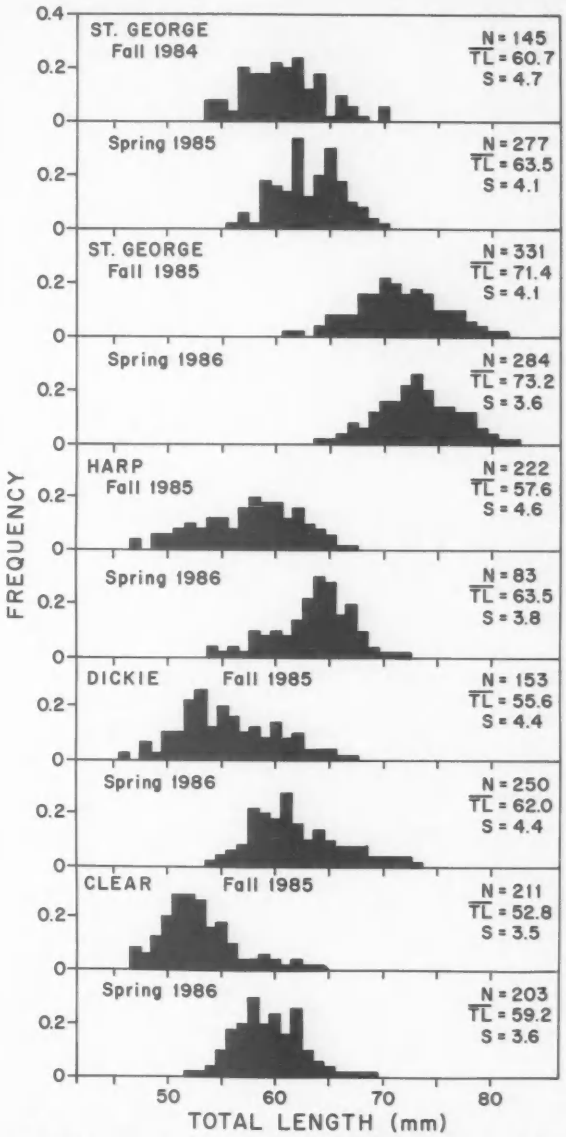
ST. GEORGE Fall 1984 N = 145 TL = 60.7 S = 4.7
Spring 1985 N = 277 TL = 63.5 S = 4.1
ST. GEORGE Fall 1985 N = 331 TL = 71.4 S = 4.1
Spring 1986 N = 284 TL = 73.2 S = 3.6
HARP Fall 1985 N = 222 TL = 57.6 S = 4.6
Spring 1986 N = 83 TL = 63.5 S = 3.8
DICKIE Fall 1985 N = 153 TL = 55.6 S = 4.4
Spring 1986 N = 250 TL = 62.0 S = 4.4
CLEAR Fall 1985 N = 211 TL = 52.8 S = 3.5
Spring 1986 N = 203 TL = 59.2 S = 3.6
FREQUENCY
TOTAL LENGTH (mm)

FIG. 9. Fall and spring length-frequency distributions of young-of-the-year yellow perch in the study lakes. Sample size (N), mean total length (TL) in mm and standard deviation (s) are indicated.

winter of 1985–86. We therefore estimated a distribution of winter durations for Clear Lake from the observed winter durations for Harp and Dickie lakes pooled plus 7 d (mean = 170.8 d, SD = 8.3 d, $N = 20$ yr). Interannual variation in winter duration did not differ amongst our study lakes (Bartlett's test, $B = 1.01$, $P>0.05$) and was therefore pooled (SD = 8.6 d).

Annual variability in fall mean lengths was not estimated for our study lakes but was estimated from published data for Red Lakes, Minnesota, and Oneida Lake, New York where the standard deviations of fall mean lengths were 9.07 mm (mean = 69 mm, $n = 20$ yr) and 8.03 mm (mean = 68 mm, $n = 11$ yr), respectively (Pycha and Smith 1955; Ney and Smith 1975; Nielsen 1980). Annual variation in fall mean total lengths for our study lakes was assumed to be the same as those for Red Lakes and Oneida Lake which did not differ significantly (variance ratio test, $F = 1.27$, $P>0.05$) and were therefore pooled (SD = 8.7 mm).

The simulation model included four procedures: (1) 1000 winter durations were randomly selected from a normal distribution of winter durations with a lake specific mean and a standard deviation pooled across lakes, (2) 1000 fall mean total lengths were randomly selected from a distribution with a cohort specific mean and a pooled standard deviation from the Red Lakes and Oneida Lake data sets, (3) a fall length-frequency distribution of 100 individuals was created by sampling at random from a normal distribution with the mean total length selected in the second step and cohort specific standard deviation, and (4) overwinter mortality was calculated using the laboratory-derived starvation model using the simulated winter

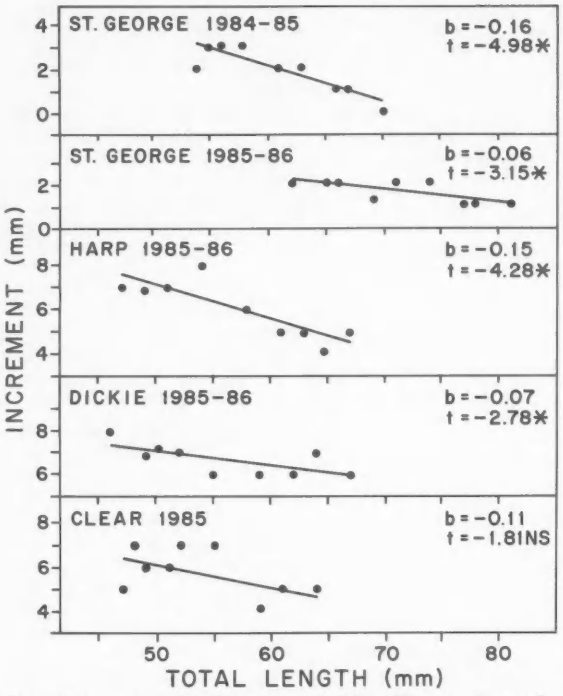

FIG. 10. Increment plots from the study lakes. Slopes and *t*-test for the hypothesis that $b \geq 0$ are indicated. Significant ($P<0.05$) negative slopes are indicated by *.

durations and fall length frequency distributions. The model was run for 1000 pairs of randomly selected length-frequency distributions and winter durations for each of the five cohorts for which we collected empirical data.

Stochastic variation in body size of YOY yellow perch and in winter duration within empirically observed bounds is sufficient to produce substantial variation in year-class strength (Fig. 11). Overwinter mortality in all populations varied from 0 to 100% during the 1000 yr simulated. Mean annual mortality varied from 20% for the 1985–86 Lake St. George cohort, with the shortest mean winter duration and largest body size, to 63% for the 1985–86 Clear Lake cohort with the longest mean winter duration and smallest body size. Variability in overwinter mortality was lowest for the 1985–86 Lake St. George cohort with the largest mean body size (Fig. 11). We conclude from these simulations that overwinter starvation mortality can produce substantial variability in year-class strength of yellow perch in lakes which have first year growth rates and winter durations similar to our study lakes. Lakes at more northerly latitudes experiencing longer winters and supporting lower growth rates would be expected to experience greater overwinter starvation mortality and more intense size-selective mortality than observed in the lakes we studied.

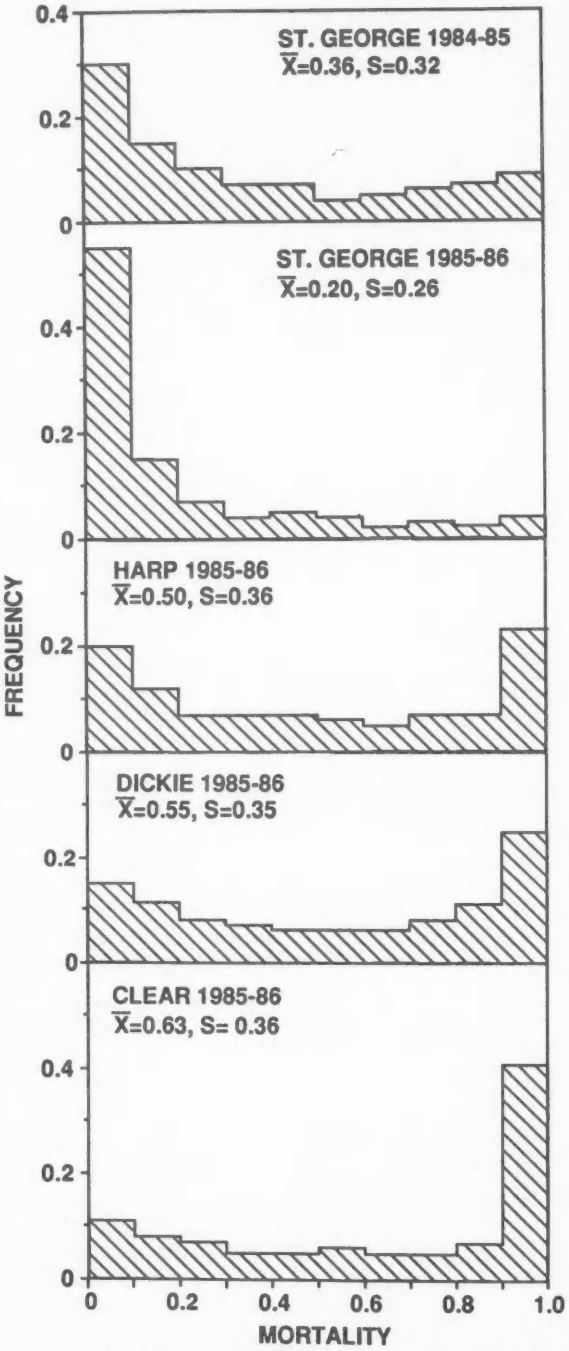

FIG. 11. Predicted frequency distributions of overwinter survival from 1000 yr of simulated length-frequency distributions and winter durations. The simulations used cohort specific empirical mean total lengths, lake specific mean winter durations, and pooled standard deviations of mean total lengths and winter duration. Mean and standard deviation of simulated overwinter survival are indicated for each cohort.

Discussion

The physiological mechanism hypothesised to explain size-dependent overwinter mortality is based on metabolic allometry. Smaller fish, with lower absolute energy stores and relatively higher metabolic rates (Paloheimo and Dickie 1966), would be expected to have higher mortality rates during winter

when energy stores are metabolized and the fish can not meet metabolic demand. Field and experimental evidence for YOY fish supports this contention. Immature yellow perch in the field in the spring had approximately one half the fat content that they had in the summer, expressed as percent of dry weight (Newsome and Leduc 1975). YOY smallmouth bass showed a significant overwinter decrease in wet weight and ash-free dry weight in both the field and in laboratory experiments (Cunningham and Shuter 1986). YOY yellow perch, largemouth bass, brook trout, and smallmouth bass all showed reductions in fat content as a percent of dry weight when held in the laboratory under simulated winter conditions (Toneys and Coble 1980). These field and laboratory studies all suggest that YOY fish utilize stored energy reserves through the winter. Shuter et al. (1980) have modeled in detail the size-specific loss of energy stores for smallmouth bass and the implications for overwinter survival. In our laboratory experiment, the loss of body weight was also proportionately higher for smaller individuals in both the fed and starved treatments.

Laboratory evidence of size-related overwinter mortality of YOY fish has been demonstrated for yellow perch (Toneys and Coble 1980) and for smallmouth bass (Oliver et al. 1979). In these studies, surviving fish were on average larger than were fish that died through the course of the simulated winter and also, for smallmouth bass, the rate of mortality increased with simulated winter duration. Our laboratory model for YOY yellow perch of the dependence of mortality on body size and winter duration is qualitatively similar to that presented by Shuter et al. (1980) in their analysis of smallmouth bass data of Oliver's et al. (1979). Since the implied mechanism in our model is the exhaustion of stored energy, we would expect that the model would only be applicable to the specific range of conditions for the population used in the experiment. In five southern Ontario lakes including Dickie Lake, the source of fish for this experiment, there were no significant differences in condition in the fall of 1985 (Post 1987). Therefore, the model we present may be generally applicable to other populations of yellow perch.

Results from field studies of overwinter mortality are less clear. Changes in length-frequency distribution and or increases in mean lengths of subsamples from natural and experimental populations from fall to spring were interpreted to be a result of size-selective overwinter mortality in walleye (*Stizostedion vitreum vitreum*) (Forney 1966, Chevalier 1973), sander (*Lucioperca lucioperca*) (Negonovskaya 1972), smallmouth bass (Shuter et la. 1980), bluegill and largemouth bass (Toneys and Coble 1979). Evidence of size-selective overwinter mortality was not found for bluegill, fathead minnow (*Pimephales promelas*), white crappie (*Pomoxis annularis*) or green sunfish (Toneys and Coble 1979). The lack of definitive field evidence of size-selective mortality from sequential length frequency distributions should not be surprising since Ricker (1969) demonstrated that size-selective mortality, if a linear function of size, would not significantly alter the shape of length-frequency distribution s. Unless the mortality-size function was extreme, the effect of mortality on mean length would also be small. In our laboratory study, which generated a distinctly nonlinear mortality-size function, fall and spring length-frequency distributions did not differ significantly, yet mortality was strongly size-related.

A second factor that complicates inferences about size-selective mortality from sequential length-frequency distributions is growth. Limited growth during late fall to early spring was apparent for the five natural yellow perch cohorts that we studied and also for the fish in the enclosure experiment. Empirical quantile–quantile plots (Chambers et al. 1983), however, can be used to examine the countervailing effects of size-dependent mortality and growth. Furthermore, the technique provides a quantitative measure of the intensity of size-selective mortality if the size-dependence of growth is known. Previous field studies (Toneys and Coble 1979; Shuter et al. 1980) provided only a visual estimation of the occurrence and intensity of size-selective mortality. Application of the QQ technique to the five cohorts of YOY yellow perch assessed in this study identified the occurance of overwinter size-selective mortality favouring the survival of the larger individuals.

From the perspective of the population, the critical factor is the total overwinter mortality which is determined by the intensity of size-selective mortality. In Oneida Lake, New York, total mortality of 11 cohorts of young-of-the-year yellow perch ranged from 72 to 99% from October 1 to the following May 1, as determined from relative abundance estimates (Nielsen 1980). Reanalysis of overwinter total mortality (age-0, October 15 to age-1, May 1) and mean length data (October 15) presented in Tables 1 and 7 from Nielsen (1980) yielded a significant negative relationship ($r = -0.68$, $df = 9$, $P < 0.05$) (Post 1987). Although Nielsen (1980) attributed mortality of juvenile yellow perch, during the growing season, to predation by walleye, overwinter mortality (October 15 to May 1) of YOY yellow perch was not positively related to walleye biomass ($r = -0.32$, $df = 9$, $P > 0.05$) (Post 1987). Size-selective starvation may be the main cause of the observed overwinter mortality. In Lake St. George, total overwinter mortality was 76% for the small-bodied 1984 cohort and 42% for the larger-bodied 1985 cohort. Similarily, overwinter mortality ranged from 27 to 65% for wild fingerling brook trout during 11 successive winters in Lawrence Creek, Wisconsin, and was also negatively correlated to body size (Hunt 1969). In summary, field studies identify substantial total mortality during the winter, and indicate that survival is higher for cohorts that attain a larger body size by the end of their first growing season.

The results from a stochastic simulation model suggests that substantial annual variability in overwinter mortality and hence year class strength could be caused by the combination of variability in first year growth and climate. Our study lakes included populations in which overwinter starvation mortality was low (St. George 1985 cohort) to those in which overwinter mortality may be sufficient to limit abundance (Clear 1985 cohort). We suggest that the variance in year-class strength attributed to overwinter starvation mortality is maximal at latitudes characteristic of southern Ontario. We would predict that, at more southerly latitudes with longer growing seasons and shorter winters, that overwinter starvation mortality would be low and less variable. At latitudes north of our study lakes with lower productivity and longer winters we would predict that overwinter starvation mortality would be higher and less variable.

Wide fluctuations in the abundance of year-classes of yellow perch and the Eurasian perch have frequently been noted in the literature (Nielsen 1980; Craig and Kipling 1983; Henderson 1985). Nielsen (1980) presented a multiple regression model that explained a significant amount of the variance in egg to age-2 mortality over 12 yr in Oneida Lake. One of the three independent variables was YOY yellow perch mean length at the end of the first growing season. Craig (1982) and Craig and Kipling (1983) presented a multiple regression model that

explained a significant amount of the variation in perch recruitment in Lake Windermere. They identified temperature, expressed as accumulated degree days over 14°C over the first summer of life, as a significant variable. Their temperature index was positively correlated to first year growth. Therefore, both models identified first year growth as significantly correlated to the abundance of recruits. Size-related overwinter mortality is one mechanism that would produce this correlation. Year-to-year variation in first year growth within a population of yellow perch can be substantial (Pycha and Smith 1955; Ney and Smith 1975; Nielsen 1980; Mills et al. 1987). Abundant YOY yellow perch can suppress their own preferred food resource, leading to utilization of suboptimal prey sizes, producing density-dependent growth (Mills et al. 1987; Post and McQueen 1987). Therefore, in years when YOY yellow perch, or interspecific competitors are abundant, first year growth may be suppressed, increasing the probability of overwinter starvation mortality especially if a severe winter should coincide with a cohort of small-bodied fish.

The stochastic variation in winter duration, which was not included in either of the Oneida or Windermere perch models, would be expected to add significant variability to year-class strength, unexplainable by biological variables. Size-dependent overwinter mortality and the stochastic variation in winter duration may provide a mechanistic explanation for the observed importance of YOY size to year-class success and recruitment in perch, and may substantially reduce the unexplained variance in existing recruitment models.

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Value of Short-Term Forecasts of Recruitment Variation for Harvest Management

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The expected improvement in management performance due to using short-term forecasts can be assessed with stochastic dynamic programming. Numerical studies on test cases indicate that this improvement depends strongly on average productivity and on the flexibility of the inseason regulatory system that is used to adjust stock size estimates and target harvests within each year. For productive stocks that are managed with flexible inseason systems, such as larger Pacific salmon stocks, only modest improvements in average yield (5–10%) can be expected from improved forecasting. Very large (30–50%) improvements would be possible in principle for such stocks if they were managed with inflexible annual quotas set before each fishing season (as would happen for example under some proposals for improving economic performance through vessel quotas), but only if perfect preseason forecasting were practical. For unproductive stocks (where harvest rates are always low), only modest improvements (5–15%) can be expected from short-term forecasting, no matter what inseason regulatory system is used.

On évalue, par la programmation dynamique stochastique, à quel point les prévisions à court terme peuvent améliorer la gestion des stocks de poissons. Les résultats d'expériences numériques révèlent que l'amélioration de la gestion est étroitement liée à la productivité moyenne et au degré de souplesse des mesures de réglementation des prises pendant la saison de pêche pour la modification des estimations des effectifs et des prises cibles. Dans le cas des stocks productifs, tels les importants stocks de Saumon du Pacifique, gérés au moyen de systèmes souples permettant d'intervenir en cours de saison, l'amélioration du système de prévision n'entraîne qu'une faible augmentation du rendement moyen, soit de 5 à 10 %. En principe, l'amélioration des prévisions devrait pouvoir entraîner de très fortes augmentations du rendement moyen, de 30 à 50 %, si les contingents annuels étaient fixés définitivement avant chaque saison de pêche – comme ce serait le cas par exemple si l'on adoptait certaines propositions visant à accroître le rendement économique par l'établissement de contingents par bateau-, mais seulement si les prévisions d'avant-saison étaient parfaites. Pour ce qui est des stocks improductifs (donnant toujours lieu à de faibles captures), les prévisions à court terme ne peuvent se traduire que par une faible amélioration du rendement moyen, à savoir de 5 à 15 %, quelles que soient les mesures d'intervention prises pendant la saison de pêche.

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Improved survey methods and research on recruitment mechanisms are likely to result in better capability for forecasting short-term recruitment variations in major fish stocks. It has been taken for granted that this capability will result in opportunities for improved biological management, through planning to take advantage of strong recruitment events and to protect stocks when poor recruitments are expected. However, there has been little research on precisely how harvests should be varied in anticipation of recruitment variations, and on how much improvement in long-term or average performance can be expected from making anticipatory adjustments in harvest policies.

Existing theory on optimal responses to environmental variation has mainly been developed under the assumption that there is some knowledge about the distribution of variations (average stock-recruit relation, spread of recruitment rates around this average), and that the "manager" has access each year only to this distribution and to an estimate of the current stock size (which reflects the cumulative effects of past variations and policy choices). Under these conditions, the optimal harvest decision each year will be some feedback function of the stock size estimate available at the start of the year, perhaps adjusted within the year through an inseason regulatory process; the optimal feedback function can be computed using methods such as stochastic dynamic programming. Generally it is found that the optimal feedback function is relatively simple; for example, a fixed escapement or fixed base stock policy (harvest excess above the fixed base each year) will generally result in maximum long-term catch. For reviews of this theory and computational methods, see Clark (1985), Mangel (1985), and Walters (1986). It is not surprising that the theory has led to recommendations of "reactive" feedback policies based only on current state and past distributional information, since the possibility of predicting specific short-term variations (and hence of using "anticipatory" policies), has been ignored in the theoretical formulations.

This paper examines how harvests should be varied when the management system has available at the time of decision each year not only information about stock size and the long-term distribution of recruitment variations, but also a specific forecast of the next recruitment variation that will occur in the stock. This forecast may come from a variety of sources, such as pre-

recruit surveys or correlations between recruitment and environmental factors. Two strategies are examined depending on when the annual forecasts become available and whether there is inseason flexibility to adjust stock estimates and catches. In the first strategy, there is a flexible inseason system to update stock size estimates so as to accurately reach a target escapement (or target base stock) after harvest each year, and at the start of each season the manager has a forecast of the relative productivity that will be achieved by each spawner after the season (i.e., of next year's stock size). In the second strategy, the manager knows only last year's spawning stock, and must set a quota for this year based on that spawning stock and on a forecast of how productive it has been in generating the stock size from which the quota will be taken. For each strategy, two stock types are examined: a highly productive stock with recruitment parameters similar to B.C. sockeye salmon (*Oncorhynchus nerka*), and a less productive stock with parameters similar to Pacific halibut (*Hippoglossus stenolepis*). For each strategy/stock combination, stochastic dynamic programming is used to estimate optimal feedback policies and expected yields for a range of accuracies of recruitment forecasts. By examining a range of forecasting accuracies, it is possible to determine whether even inaccurate forecasts should be used in anticipatory policies; it is conceivable that using inaccurate forecasts would be worse than using a simpler reactive policy in the first place. It is assumed in all calculations that the basic management objective is to maximize total long-term yield; under this objective the optimum policy will generally be to allow a fixed escapement each year, rather than some simpler policy such as $F_{0.1}$ that might be more forgiving of forecasting errors.

The utility of stock-recruitment models for management has been widely misunderstood by fisheries scientists. It is often claimed that such models are (or should be) ignored by managers because they are usually almost useless for short term forecasting due to the wide scatter of recruitment observations around any average curve (for a recent survey and analysis of this claim, see Fletcher and Deriso 1988). The misunderstanding is in failing to distinguish between the use of stock-recruit analysis to define long-term strategies (target stock levels, feedback policies) versus short-term forecasts and tactical responses. In the cases examined below, recruitment rates are assumed to vary so widely around the average stock-recruit curve as to make the curve almost useless for short-term forecasting; in spite of such variability and even given an alternative scheme for short-term forecasting, the average curve remains important for estimating the optimal long-term strategy.

Optimization Models for Using Recruitment Forecasts

This section begins with a simple review of stochastic dynamic programming as a method for finding optimal harvest policies in variable environments. It then describes the forecasting, regulation, and population response models used in numerical tests of forecasting value.

To specify an optimal policy for managing harvests in a variable environment, it is necessary to first define two key things: (1) a dynamic model for stock response to harvest and to uncontrolled variation; and (2) a management performance measure or objective function. Dynamic models for stock response can generally be written as

$$(1)\quad N(t+1) = f[S(t), w(t)]$$

where $N(t+1)$ is a measure of stock size (numbers or biomass) at the start of harvesting in year or generation $t+1$ (the measure may be a single scalar, or vector representing age structure, etc.). $S(t) = N(t) - C(t)$ is the stock size in year t after removing harvest $C(t)$ ($S(t)$ is usually referred to as the "spawning stock" even if there is some loss of fish between the time of harvest and the time of spawning), and $w(t)$ is a random "environmental effect". The simplest way to think of equation (1) is as a family of stock-recruitment or surplus production response curves, with each curve generated by a different value of $w(t)$. There can also be a model for the behavior of $w(t+1)$ in relation to $w(t)$, for example to represent serial correlation in environmental effects; for simplicity, I will assume in what follows that the $w(t)$ are independent random effects.

Stochastic dynamic programming assumes that management performance can be measured as a sum $V(t)$ of annual rewards or net benefits $v(t)$ over a finite planning horizon t to T:

$$(2)\quad V(t) = v(t) + v(t+1) + \ldots + v(T)$$
$$= v(t) + V(t+1)$$

Note here that $V(t)$ is a random variable, due to stochastic effects on the $v(t)$ associated with uncontrolled changes in $N(t)$. It is also dependent on the system state $N(t)$ at the time of decision; to simplify the notation in the following presentation, this important functional dependence will be left out of the equations though it is critical in computational schemes. I will assume that the management objective is to maximize the expected value of $V(t)$ over possible stochastic outcomes. The planning horizon T may be made very large, and/or used in conjunction with discounting in the calculation of the $v(t)$ in order to represent an infinite planning horizon; essential conclusions about optimal policy and value are not sensitive to the choice of T provided $T>10$ for the models discussed below.

A variety of short-term benefit measures $v(t)$ have been used in harvest policy analysis. For simplicity in assessing the relative value of forecasting, I will use the simple measure

$$(3)\quad v(t) = N(t) - S(t)$$

which will represent the total catch taken in year t. With this measure, $V(t)$ represents the expected cumulative long-term catch (the stochastic version of sustainable yield).

To find an optimal policy with stochastic dynamic programming, the problem variables $N(t)$, $S(t)$, $w(t)$ and any policy variables are first divided into a set of discrete levels, and an arbitrary terminal value $V(t)$ at time T. Then beginning at time T-1, the maximum value $V(T\text{-}1)$ that can be achieved for each discrete state $N(T\text{-}1)$ that might have arisen by time T-1 is found by testing alternate policy choices so as to find the choice that maximizes the expected value of the sum $v(T\text{-}1)+V(T)$. This "backward recursion" (Bellman 1957) is then repeated for time T-2, using the $V(T\text{-}1)$ just computed to find best values of $v(T\text{-}2)+V(T\text{-}1)$, then for T-3 on back to $t=1$. This computational procedure is essentially very simple but tedious when the state is finely discretized. It can be accomplished in a few minutes on a personal computer with a short program provided there are only one or two state measures discretized at 100 or fewer levels each. To avoid numerical errors and artifacts due to discretization, the computation is repeated for progressively finer discretizations (more state and policy variable levels) until essentially the same answers are obtained. For further discussion of discretization problems and efficient computational algorithms, see Ludwig and Walters (1982), Mendelssohn

(1980a, b), and Mangel and Clark (1988). The discretization procedure used in this paper is described in more detail in Appendix A.

It is usually found that the best policy choice for each discrete state level becomes independent of time after the first or a few backward steps T-1, T-2,. . ., depending on the choice of terminal value function $V(T)$. We say then that an "optimal stationary policy" has been found, in the form of a functional relationship between the best policy choice and the state.

Information States and Forecasts of Recruitment Variation

In the simplest applications of stochastic dynamic programming, it is assumed that only the state $N(t)$ (or an estimate of it) is available at each decision time, and that the manager should make whatever decision choice will maximize the expected value $v(t)+V(t+1)$, where the expectation is calculated using probabilities of alternative outcomes $w(t)$. The stock model (equation 1) is used to specify $N(t+1)$ given $N(t)$,$w(t)$ and any policy choice, so that averaging over different $w(t)$ outcomes (with associated probabilities) is translated into an averaging over N(t + 1) values where a different value $V(t+1)$ has already been calculated for each $N(t+1)$. $N(t)$ is called the "information state" available to the manager at time t for assessing probabilities of different outcomes $N(t+1)$.

It is not necessary to assume that only $N(t)$ is available to the manager for making his assessments of expected value. The general requirement is that he be able to assign probabilities to alternative information states at time $t+1$, where the probabilities are assessed from his information state at time t. Indeed, he may not even have a reliable estimate of $N(t)$. His information state may consist of $S(t$-1), a prediction of $w(t)$, and/or other measures that allow him to place odds on alternative information states at time $t+1$.

Suppose that the manager's information state at time t includes only only the stock size $N(t)$, but also a prediction $y(t)$ of the stochastic effect $w(t)$ that will influence (along with harvesting) the recruitment rate leading to the next stock size $N(t+1)$. To make use of this prediction in calculating the expected value of any policy choice, two probabilistic assessments are needed about $y(t)$: (1) a probability distribution for the actual (realized) effect $w(t)$ given the prediction (i.e., how probable are different values of $w(t)$ given a prediction of it); and (2) a probability distribution for the next predicted effect $y(t+1)$. Given the information state $\{N(t),y(t)\}$, the first probability distribution is needed to assign odds to different values of $N(t+1)$ in the information state $\{N(t+1), y(t+1)\}$ for time $t+1$, and the second distribution assigns odds to different $y(t+1)$ in that next information state.

Now suppose historical data indicate that $w(t)$ values are likely to follow the probability distribution $P(w)$, and that evaluation of the forecasting method using historical data suggests a distribution P' $(y|w)$ of values $y(t)$ conditional on (or distributed around) the actual values $w(t)$. At least some information about the P′ distribution can be obtained by noting that for an unbiased forecast, $E(y|w)=w$ and var $(y|w)=v(1-R)/R$ where v is the variance of the $P(w)$ distribution and R is the square of the correlation coefficient between the predicted and observed environmental states (note that in order for there to be a zero correlation, var $(y|w)$ would have to be infinite since $R=1-\text{var}(y|w)$ /var(y) and var (y) = var (w) + var $(y|w)$. For expected value calculations, we need the marginal distribution P'' (y) of the $y(t)$ values and the conditional distribution $P^*(w|y)$ of actual outcomes given predictions (see Appendix A). The marginal distribution $P''(y)$ is easily found for each y as the sum (or integral) over w values of $P(w)P(y|w)$. The distribution P^* is given by Bayes theorem as

$$(4)\quad P^*(w|y)=P'\ (y|w)P(w)/P''\ (y).$$

Notice here that the probabilities assigned to different values of w conditional on any forecast value y will depend strongly on the forecast value y only if P' assumes a tight distribution (high R) of y values for each w (high forecast accuracy). If P' assumes a wide spread of y for each w, then P^* will depend mainly on the overall, natural ("prior") distribution $P(w)$ of the environmental effects.

Alternative Regulatory Systems

Effects of forecasting on management performance cannot be evaluated without specifying with some precision what other information the manager has available at the time when harvesting decisions are made, and how much flexibility he has to vary these decisions in the face of new information as fishing proceeds. I have based evaluations on two extreme situations. At one extreme is an "ideal" system where inseason management involves a whole series of fishery openings and monitoring opportunities, so that by the end of each season the manager can reliably estimate the total stock size $N(t)$ and vary catches so as to reliably reach any post-harvest stock size target $S(t) <= N(t)$ that may be optimal for year t; below I refer to this extreme as the IA (Inseason Adaptive) situation. At the opposite extreme is a potentially "pathological" system where the manager has a reliable estimate of the stock size after harvest last year, and must set a quota this year based on that estimate without opportunity for inseason adjustment except to prevent the quota from exceeding 90% of this year's stock size; below I refer to this extreme as the AQ (Annual Quota) situation.

The IA and AQ situations lead to different decision variables and information states in terms of the dynamic programming formulation reviewed above. Also the dynamic programming expected values are calculated differently (Appendix A).

In the IA situation, the manager's information state for year t is $\{N(t), y(t)\}$, and his decision variable is the escapement $S(t)$ to allow; $y(t)$ is a prediction of the recruitment deviation $w(t)$ that the recruits produced by $S(t)$ will experience before recruiting to next year's population $N(t+1)$. The realized catch performance in year t for the IA system will then be $v(t) = N(t) - S(t)$ if $N(t)>S$(t), and 0 for stock sizes below $S(t)$; the realized escapement will be the lesser of the target $S(t)$ or $N(t)$. This performance calculation assumes that the inseason management system can react very quickly to insure that only negligible catches are taken in the event that preseason forecasts have predicted $N(t)$ to be larger than the target $S(t)$.

In the AQ situation, the information state at time t is $\{S(t-1), y(t-1)\}$, and the decision variable is the "target" quota $C(t)$; here $y(t-1)$ is a prediction (perhaps by a preseason recruitment survey) of how well the spawners $S(t-1)$ did at producing recruits to this year's (unknown at decision time) population $N(t)$. The realized catch performance for the AQ system in year t will then be the lesser of $C(t)$ and $0.9N(t)$; the realized escapement $S(t)$ will be the larger of $N(t)-C(t)$ and $0.1N(t)$. Notice here that $v(t)$ must be calculated as an expected value over $N(t)$ possibilities, with the expectation including probabilities of $N(t)$ values such that $0.9N(t)<C(t)$.

As the IA and AQ systems are defined here, the IA system is expected to give better performance (long-term catch) than the AQ system, except in the limiting comparison of an IA system with zero forecast accuracy ($R=0$) to an AQ system with perfect forecasts ($R=1$). That is, the worst case for the IA system corresponds to having only $N(t)$ as the information state variable ($y(t)$ estimate giving no information about $w(t)$), while the best case for the AQ system corresponds to having exactly this same information state $N(t)$ through having a perfect prediction of $N(t)$ from $S(t-1)$ and $w(t-1)$ while again having no information about $w(t)$.

Case Models for Stock Dynamics

The relative value of forecasting may depend strongly on biological productivity and longevity. In particular, we might expect very productive stocks to be much more forgiving of forecasting errors and/or poor inseason management systems. On the other hand, it may be unnecessary to have complicated inseason management for unproductive, long-lived stocks for which target harvest rates will always be low. To examine the balance of these factors, I carried out dynamic programming calculations for two extreme cases: a sockeye salmon stock with productivity parameters similar to the Fraser river stock complex (Walters and Staley 1987; Collie and Walters 1987), and a long lived stock with parameters similar to the Pacific halibut (Quinn et al. 1985, Deriso et al. 1986).

For the productive sockeye salmon case, the population dynamics model relating $N(t+1)$, $S(t)$, and $w(t)$, with time in units of 4-yr cycles, is taken to be the stochastic Ricker equation

$$(5a)\quad N(t+1) = S(t)\exp\{1.9(1-S(t)/P_e) + 0.9\,w(t)\}$$

where $w(t)$ is assumed to be normally distributed with mean 0.0 and variance 1.0. P_e is the average unfished stock size in the absence of stochastic effects, chosen so that unit changes represent the dynamic programming discretization of N and S (e.g., the dynamic programming computation was set up so that $P_e=100$ would give 100 discrete stock levels between 0 and the unfished average stock size). The value 1.9 for the Ricker productivity parameter implies that the best average harvest rate would be around 70% of $N(t)$ each cycle. The value 0.9 for the standard deviation of environment effects represents an average over the estimates from historical data for various sub-stocks in Walters and Staley (1987).

For the halibut case, the population dynamics model was taken to be

$$(5b)\quad N(t+1) = 0.8S(t) + aS(t)/[b+S(t)]\exp\{w(t)\}$$

where N(t) is in biomass units. The term 0.8S(t) represents combined survival and growth of the fish $S(t)$ that escape harvest in year t, under the (weak) assumption that exploitation rate will remain relatively steady so that average age and body size of these fish is nearly constant (see Schnute 1987 for a justification of this formulation in terms of delay-difference models for population age structure). a and b are scale-dependent parameters of a Beverton–Holt type model $r=aS/(b+S)$ for the stock-recruit relation; if the maximum recruitment parameter a is around 20 and the stock size needed to achieve $a/2$ is taken to be $b=20$, then eq. 5b will predict an unfished average stock size ($S(t)=N(t)$) of around 100 units (solution to $N=.8N+r$ with r close to $a=20$). Equation 5b predicts significant recruitment overfishing for $S(t)<40$. The exponential term represents a multiplicative, log-normal stochastic effect on recruitment if w(t) is taken to be normally distributed; with a standard deviation of 1.0 for $w(t)$, the recruitment rate will vary widely with most (95%) of the values between 30 and 500% of the average predicted from the Beverton–Holt curve.

Note that eq. 5b ignores the 6–10-yr delay from spawning to recruitment in the Pacific halibut; including this delay would greatly complicate the optimization calculations. Using a lag of 1-yr will not change any basic conclusions about the value of short term recruitment forecasts, and would even be realistic for long-term prediction in situations where the stock size changes slowly enough so that $S(t-8)$ and $S(t)$ are strongly correlated. The lag may of course be important for generating forecasts in the first place, by creating opportunities for better estimation of S and for predicting recruitment strength from prerecruit surveys and partial recruitment information.

Computational Considerations

A basic computational technique of stochastic dynamic programming is to build up an estimate of the value function $V(t)$ over a grid of discrete information state values (combinations of $\{N(t),y(t)\}$ values or $\{S(t-1),y(t-1)\}$ values) by using the backward recursion (also called "value iteration") procedure reviewed above. The computation is simplest for the cases considered here if the V function can be stored as an array $V(i,j)$, where i is an integer value for one information state variable (i.e., $N(t)$ or $S(t-1)$), and j is an integer value or index for the second information state variable ($y(t)$). Accordingly, I scaled the population model parameters so as to give 50–200 discrete, integer levels of N, S, and C, and rounded the model equation (5a,b) predictions of N from S and w to the nearest integer in the range considered. I discretized $w(t)$ and $y(t)$ as 11 levels, corresponding to 1/2 standard deviation values of $w(t)$ from -2.5 to $+2.5$ standard deviations around the mean of 0 (similar results are obtained with even coarser discretizations, such as 5 and 9 levels). I then represented the conditional distributions P', P^*, and P as tables of discrete probabilities (e.g., $P'(i,j)$ is the probability of estimating index level i for the predicted environmental state $y(t)$, given index level j for the actual environmental state $x(t)$). To avoid the discretization problem of having $N(t)=0$ act as an "absorbing state" (Mendelssohn 1980b), I constrained the $N(t)$ predictions (from $S(t-1),w(t-1)$) to be 2 or larger.

To represent forecasting schemes with different accuracies, I constructed alternative $P'(y|x)$ tables with (1) var $(y|x)=v(1-R)/R$ where v (1.0) is the variance of $w(t)$ and R is the square of the correlation coefficient between $y(t)$ and $w(t)$; and (2) y normally distributed except with probabilities of zero for more extreme values than 2.5 standard deviations. This method of constructing the P' tables leads to slightly higher precision in forecasts, especially for extreme $w(t)$ values, than would be indicated by the R value alone. It also presumes a bias toward failing to predict extreme events, in the sense that $E(y|x)$ is smaller in absolute value than x, especially for extreme x values. In fact, it is likely that forecasting and prerecruit survey schemes will tend to underestimate the severity of extreme events, so truncation of the tables is probably not unrealistic.

By experimenting with different discretization schemes (50 vs 100 vs 200 levels for N, etc.), I found for both test models that essentially the same results were obtained whenever the discretization was fine enough to have more than 100 $N(t)$ levels between 1 and the unfished equilibrium. $N(t)$ values far above equilibrium are probable in at least the salmon case, so I ended

up having to compute the value function for up to 300 discrete $N(t)$ levels.

Indices for Comparing Performance

By repeating the backward recursions for different time horizons (T) from 10 to 30 yr (cycles for sockeye case), I found that the predicted relative performances for different forecasting accuracies are not sensitive to the time horizon, so all comparisons presented below are for a $T=10$ (relatively short) time horizon. More precisely, suppose that $V^*(i',j')$ is the computed value function for $t=1$ at some probable or typical index states i' j', for a calculation carried out with an identity matrix for P' (i.e., perfect forecasts with $y(t)$ having a probability of 1.0 of equalling $w(t)$). Suppose that $V'(i',j')$ is the same value function table element, except computed with a P' matrix whose off-diagonal elements reflect a low correlation between $y(t)$ and $w(t)$. I found that the ratios V'/V^* were stable for $T=10$ or larger.

Computing the dynamic programming value function $V(i',j')$ after T backward recursion steps is equivalent to doing a large number of T-year Monte Carlo simulations beginning at stock size i' and environmental forecast j', while following the optimal feedback policy estimated from dynamic programming; the average total catch over these simulations should equal $V(i',j')$ if enough simulations are performed. For performance comparisons in the following section, I therefore chose to use the value function assessments directly, without bothering to make simulation tests except as checks on the effects of discretization in the dynamic programming algorithm. To simplify the comparisons, I present $V(i',j')$ results only for $T=10$, i' equal to the average stock size or spawning stock expected if the optimal policy has been followed for a long time prior to $t=1$, and $j'=6$ which corresponds to a forecast of average environmental condition $w(t)=0$ or $w(t-1)=0$ for the initial decision at $t=1$. Note however that the dynamic programming calculations involve computing $V(i',j')$ for all possible stock-environment combinations i' j', not just at a single starting combination as would typically be done in simulation studies.

Effect of Forecast Accuracy on Expected Performance

Maximum expected catches over 10 cycles and effects of forecasting accuracy are strikingly different for the inseason adaptive (IA) and annual quota (AQ) regulatory systems in the sockeye salmon case (Fig. 1). Surprisingly, for the IA system there is little improvement (5–10%) in moving from having no forecasting system ($R=0$) to having a perfect forecast ($R=1$) of the relative productivity that the spawners in cycle t will exhibit (relative survival over the next 4 yr of their offspring). In contrast, there is gross deterioration in performance under the AQ system, to less than 20% of the expected performance under an IA system, as the forecasting accuracy drops from perfect to even the unrealistically high level represented by $R=0.8$. In intuitive terms, unless the AQ manager has an almost perfect forecast of $N(t)$ from $S(t-1)$ and $y(t-1)$, he needs to set extremely low quotas in order to avoid the risk of severely depleting the spawning stock $S(t)$. Figure 1 is a dramatic demonstration that Pacific salmon managers have been wise to resist various pressures from the fishing industry for stabilized quotas or firm preseason commitments to target catches each year.

One might suspect that the dramatic loss in value for the salmon AQ case under imperfect forecasts is due to using a dome shaped recruitment curve, where using lower quotas to avoid overfishing could also result in "overescapement" (spawning stocks to the right of the dome). To check this possibility, I redid the calculations with a Beverton–Holt type recruitment model $N(t)=cS(t-1)/(1+cS(t-1)/100)\exp\{w(t)\}$ where $c=\exp\{1.9\}$. The results were similar to Fig. 1, with the expected catch dropping by 70% from the $R=1$ to the $R=0.8$ calculations. The loss in value for both models is due mainly to foregoing catches in order to avoid recruitment overfishing, and the loss is only slightly exaggerated when foregone catch may result in overspawning.

Differences between expected catches for IA versus AQ regulation are much smaller for the halibut case (Fig. 2), as expected considering that year-to-year changes in recruitment will be a relatively small component of stock size variation in the first place (there is less need than in the salmon case to react quickly to recruitment variations). Again the effects of having better short term forecasts are almost unmeasurable for the IA

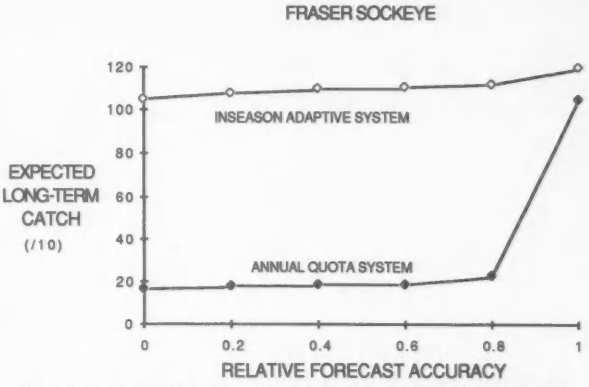


FIG. 1. Expected 10-cycle catches for Fraser River sockeye salmon, under two different inseason regulatory systems. Points along each curve show the maximum that can be obtained by following an optimal policy given the corresponding forecasting accuracy. Forecasting accuracy is defined as the square of the correlation between forecast and observed deviations from the logarithm of the recruitment relationship.

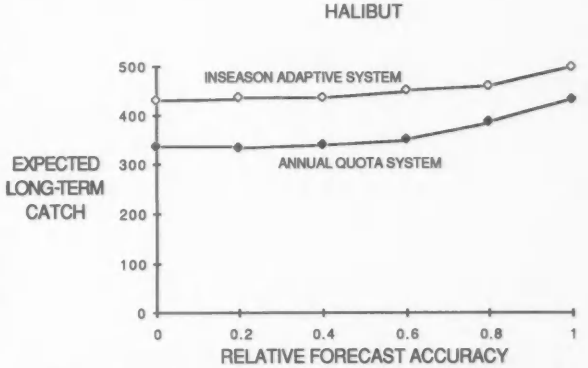
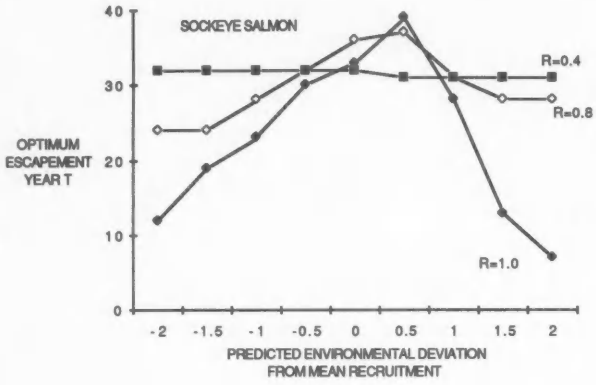


FIG. 2. Expected 10-yr catches for a hypothetical halibut population, under two different inseason regulatory systems. Points along each curve show the maximum that can be obtained by following an optimal policy given the corresponding forecasting accuracy. Forecasting accuracy is defined as the square of the correlation between forecast and observed deviations from the logarithm of the recruitment relationship.



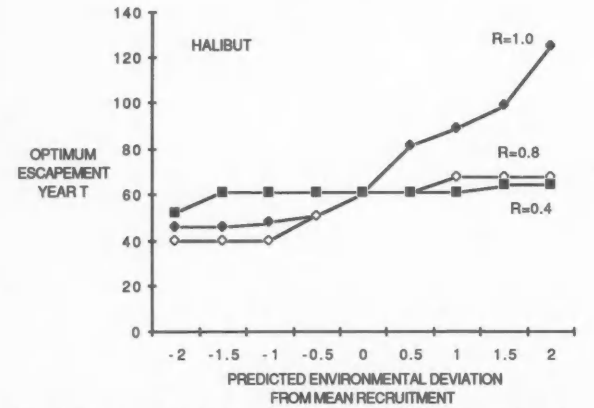
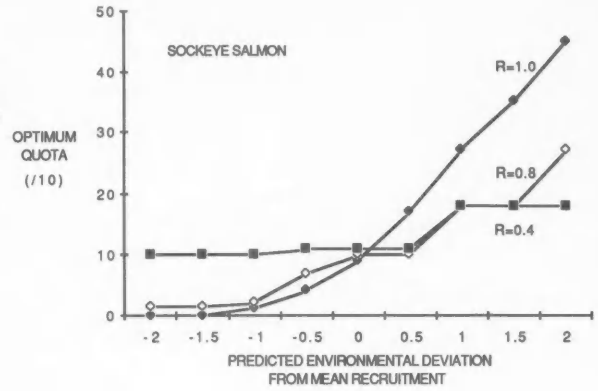


FIG. 3. Effect of forecasting accuracy on the optimal target escapement (stock size after harvest each cycle or year), for the sockeye salmon and halibut case models, when an inseason adaptive management system is used. Accuracy R is the square of the correlation between forecast and observed deviations from the logarithm of the recruitment relationship.



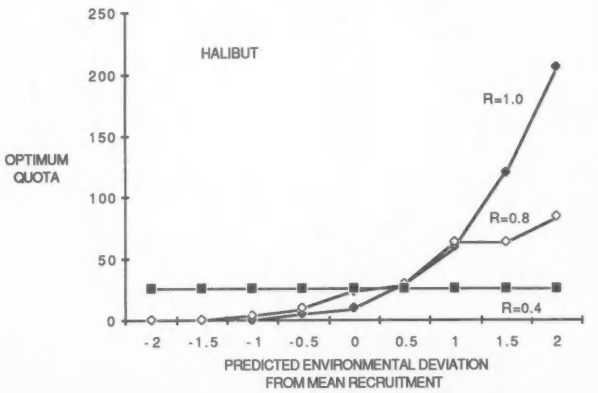


FIG. 4. Effect of forecasting accuracy on the optimal target quota for the sockeye salmon and halibut cases, when a fixed quota is set for each season without inseason updating of stock size estimates. Accuracy R is the square of the correlation between forecast and observed deviations from the logarithm of the recruitment relationship.

system, whereas moving from no forecasting to perfect forecasts could result in an improvement of around 30% in total 10-yr yield under the AQ system. Note, however, that the R value must exceed 0.6 (i.e., forecasting method explain at least 60% of recruitment variability around mean $S-R$ relation) in order for there to be any measurable improvement in performance under the AQ system.

Effect of Forecast Accuracy on Best Feedback Policy

For forecast accuracies below about $R=0.8$, the best post-harvest stock choice $S(t)$ under the IA system is essentially independent of the environmental effect forecast ($y(t)$ or $y(t-1)$) for both the sockeye and halibut cases (Fig. 3). In other words, it is best to just ignore inaccurate forecasts under IA management, except perhaps to respond weakly to extreme forecasts. Given high forecasting accuracy ($R>0.8$), the best sockeye strategy is to lower the target escapement when either very poor or very good environmental conditions are predicted; intuitively, it is unnecessary to plan for high escapements when good survival conditions are expected, and not worth trying to compensate for expected poor conditions by trying to allow more spawners. A different and less dramatic pattern occurs in the IA regulation case for halibut; it is best to adjust target escapement downward when poor environmental conditions are expected, but to allow above average escapement when unusually good conditions are expected; in other words, do not try to "stockpile" spawners when poor survival of recruits is expected, but do allow more escapement to take advantage of good survival conditions when such conditions are almost sure to occur.

Under an AQ management system, optimal quotas are sensitive to recruitment forecasts even when the forecasting accuracy is quite low (Fig. 4). For both the salmon and halibut cases, the best strategy is to adjust quotas downward when poor environmental conditions are expected, and upward when good conditions are expected. For very high forecasting accuracy ($R>0.8$), these adjustments amount to setting the quota $C(t)$ so that the spawning stock $S(t)=N(t)-C(t)$ is expected to equal its best value for maximizing long-term average catch.

No special significance should be attached to the particular numerical patterns shown in Fig. 3–4, especially for cases involving low forecasting accuracy (low R values). In low R tests, I found the predicted optimal strategies (but not the overall average performance) to be extremely sensitive to the numerical discretization scheme. The instability occurs because nearly the same expected values $v+V$ are assigned to several (or even a wide range of) policy choices, so small numerical changes in

the computation scheme can shift the optimal choice considerably. In practical terms, the expected value is relatively insensitive to the policy choice under such circumstances.

Discussion

The results presented above lead to three main conclusions about the value of short-term forecasting for harvest management performance. First, being able to forecast recruitments 1 yr (or cycle) ahead has very little effect on potential performance in situations where there is a flexible inseason management system (IA situation). The best policy given a very precise forecast is essentially to behave as though the anticipated recruitment deviation will define a new average recruitment curve for the stock. Similar results were obtained by Walters (1987) and Parma (1989) in analyses of cyclic recruitment variation.

Second, very precise short-term forecasts would allow substantially improved performance in situations where the management system lacks flexibility to quickly adjust stock size estimates and catch targets (AQ situation, year-to-year setting of TACs). The potential performance improvement is closely related to stock productivity, with greater improvements to be expected from productive stocks such as Pacific salmon. A possible reaction to this finding is to argue that more flexible inseason management systems, rather than improved forecasting, should be the top priority for productive stocks.

Third, the value of forecasts degrades very quickly with decreasing forecast accuracy, especially for productive stocks managed with annual quota policies. Indeed, the numerical examples suggest that it is better to essentially ignore the forecast (and always set very conservative quotas instead) whenever the forecasting system is expected to explain less than 60–80% of the recruitment variation. This conclusion might seem counterintuitive to someone who hopes that even crude forecasts would have some value in setting catch regulations.

To understand why the dynamic programming results are so conservative, it may be helpful to look at a particular decision situation. Suppose you are charged with setting this year's quota for sockeye salmon, and your scientific advisors come forward with a very optimistic forecast that the stock size will be double average considering brood year escapement. Even if their forecasts have been quite accurate most years in the past (so they are quite confident that the stock will at least be above average), would you trust them and set double the normal quota, or even raise the quota by 50%? The dynamic programming calculation would advise you not to trust them, essentially because the long-term loss associated with even a low probability of devastating the spawning stock will more than balance any immediate gain in catch this year if the forecast does prove correct.

It is very unlikely that forecasting models accurate enough to permit taking near biological MSY under quota management systems will become available in the foreseeable future. However, that does not imply that the search for better recruitment/environment models should be abandoned. The search can be justified on at least four valid grounds. First, there may be compelling economic or social reasons for setting predictable quotas, even if such quotas must be quite conservative; in such cases, it might be worth gambling on research aimed at high precision forecasts that would reduce the losses in yield.

Second, even crude forecasts may be useful for industrial (processing, marketing) planning in fisheries managed with IA systems that result in highly variable fishing opportunities and yields. Walters and Collie (1988) question whether public research funding is justifiable when this is the only use to which the forecasts can safely be put.

Third, the performance of inseason adaptive systems depends to some degree on the quality of pre-season stock forecasts, especially where fishing seasons are short (few opportunities for inseason monitoring and adjustment) and/or where there is a high probability of obtaining stock sizes so low that no fishery should be permitted at all. An example of quantitative assessment aimed at measuring the value of preseason forecasts in such settings is Buckingham and Walters (1975); we found that total yields of Skeena River sockeye and pink salmon (*Oncorhynchus gorbuscha*) might be improved by as much as 10–20% through better preseason forecasts of run sizes and timing, with timing being the more critical variable to predict accurately.

Fourth, research aimed at predicting recruitment deviations $w(t)$ from environmental factors may be useful in providing methods to "filter" stock-recruitment data to obtain more accurate assessments of the average (long-term) stock-recruitment relation. Accuracy in assessment of the average relationship (having the right model parameters in equations like 5a,b) is important for feedback policy design even in cases where IA systems permit timely response to random variation around the average. There are serious research difficulties in trying to untangle the effects of environment and stock size on recruitment (Walters and Collie 1988), but these difficulties are certainly worth trying to overcome.

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Appendix A: Numerical Procedure for Dynamic Programming

This appendix provides additional details on the dynamic programming procedure, for readers who may wish to try other problems. Let $n = 1,2,\ldots,N_n$ be a set of discrete stock sizes, $s = 1,2,\ldots,N_n$ be discrete escapement levels, and $c = 1,2,\ldots,N_n$ be discrete catches. Let the index $i = 1,2,\ldots,11$ represent discrete values $W(i)$ of the actual environmental effects w, with $W(1) = -2.5h$, $W(2) = -2h,\ldots$, $W(11) = 2.5h$, where h is the standard deviation of w. Let $j = 1,2,\ldots,11$ represent discrete values $Y(j)$ of the forecast environmental effect y, with $Y(1) = -2.5h$, $Y(2) = -2h$, ..., $Y(11) = 2.5h$.

Before beginning the dynamic programming calculation, construct the "next-states" matrix $N(s,i)$ for $s = 1,\ldots,N_n$ and $i = 1,\ldots,11$. $N(s,i)$ is the integer stock size n predicted by truncating or rounding the result of substituting s and $W(i)$ into the stock dynamics model (except set $N(s,i) = 2$ whenever n is predicted to be less than 2, to prevent numerical problems with "absorbing states"). For example, in the salmon case (eq. 5a) I took each $N(s,i)$ value to be the nearest integer to $s.\exp\{1.9(1 - s/100) + W(i)\}$.

Next, construct the following discrete probability tables. Letting $g(i) = \exp\{-0.5W(i)^2\}$, approximate the natural probability of getting environmental effect $W(i)$ by $P(i) = g(i)/\sum_i g(i)$. Letting $g(j,i) = \exp\{-0.5[Y(j) - W(i)]^2/\mathrm{var}(y|w)\}$, approximate the conditional probabilities $P'(j,i)$ of getting forecast $Y(j)$ when $W(i)$ is the actual environmental effect by $P'(j,i) = g(j,i)/\sum_j g(j,i)$. Compute the total probabilities $P''(j)$ of forecast $Y(j)$ as $P''(j) = \sum_i P(i)P'(j,i)$. Compute the probabilities $P^*(i,j)$ of environmental effect $W(i)$ occurring when the forecast effect is $Y(j)$ as $P^*(i,j) = P'(j,i)P(i)/P''(j)$.

For the AQ regulation system, the basic dynamic programming problem is to construct the value function table $V_t(s,j)$ for each measured escapement s last year, and forecast environmental deviation j for this year. $V_t(s,j)$ is the solution of the following "local" maximization problem once V_{t+1} has been computed (or set to a table of initial values for $t+1 = T$):

$$\text{(A1)}\quad V_t(s,j) = \max\{\textstyle\sum_i P^*(i,j)\,[c' + \sum_k P''(k)V_{t+1}(s',k)]\}$$

where the maximization is over quota choices $c = 1,\ldots,N_n$, the realized catch c' is given by $c' = \min\{c, 0.9N(s,i)\}$, the realized next stock after harvest (for this year) s' is given by $s' = N(s,i) - c'$, and the index k is over forecast environmental states for next year (j index for $t+1$). The 0.9 factor in setting c', is to constrain c' to be less than 90% of the realized stock size $N(s,i)$. Notice how tedious the calculation is for every s and j combination, and how it differs from simulating N over time: for each c that might be optimum given s and j, it is necessary to calculate a messy expected value (or average) of present plus future catches $c' + V_{t+1}$, over the probabilities $P^*(i,j)$ of getting different values of $W(i)$ given $Y(j)$ and over the probabilities $P''(j)$ of getting different forecasts as of next year.

The local maximization problem is similar for the IA management system. Here the value function table to be constructed is $V_t(n,j)$ where n is the current stock size and j is the index of the environmental deviation $Y(t+1)$ forecast to affect next year's recruitment. $V_t(n,j)$ is found as

$$\text{(A2)}\quad V_t(n,j) = \max\{n - s + \textstyle\sum_i P^*(i,j) \sum_k P''(k)\, V_{t+1}(N(s,i),k)\}$$

where the maximization is over choices $s = 1,\ldots,n$. Note again how for every n,j combination it is necessary to search over a range of s choices, and for each choice to compute a messy expected value of $n - s + V_{t+1}$. $n - s$ is the catch c obtained under choice s, and $V_{t+1}(N(s,i),k)$ is to be read as the tabled value $V_{t+1}(n',j')$ for $n' = N(s,i)$ and forecast environmental state $j' = k$.

Sublethal Copper Effects on Bluegill, *Lepomis macrochirus*, Foraging Behavior[1]

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Sandheinrich, M. B., and G. J. Atchison, 1989. Sublethal copper effects on bluegill, *Lepomis macrochirus*, foraging behavior. Can. J. Fish. Aquat. Sci. 46: 1977–1985.

The effects of four copper concentrations (5 [control], 31, 180, 1710 $\mu g\ L^{-1}$) on bluegill (*Lepomis macrochirus*) foraging behavior were examined with two separate experiments; one experiment assessing copper effects on the reaction distance of bluegill to two sizes of untreated zooplankton and one assessing copper effects on the functional response of bluegill to untreated (five tests) and treated (five tests) invertebrate prey. Prey used in these experiments were: *Daphnia pulex, D. magna* (Cladocera), *Hyalella azteca* (Amphipoda), and two sizes of *Enallagma* sp. (Zygoptera). Copper had no effect on the reaction distance of fish to zooplankton. There was a significant negative dose-response relationship for consumption rates of all untreated prey but not of treated prey groups. Prey handling time for bluegill capturing treated and untreated prey increased significantly with copper concentration and was the most consistently sensitive parameter measured. Capture efficiency by bluegill, although altered by copper for some prey types, was not as consistent a measure of toxicant stress. This study suggests that mechanistic measures are valuable indicators of toxicant effects on fish feeding behavior and that copper concentrations near the U.S. Environmental Protection Agency water quality criteria (18–28 $\mu\ L^{-1}$) may alter food consumption and reduce growth of fish in the wild.

Les effets de quatre concentrations de cuivre (5(témoin), 31, 180, 1710 $\mu g \cdot L^{-1}$) sur le comportement alimentaire du crapet arlequin (*Lepomis macrochirus*) ont été examinés au cours de deux expériences différentes, l'une mesurant les effets du cuivre sur la distance de réaction du poisson à deux types de zooplanctontes non traités et l'autre évaluant ces effets sur la réaction fonctionnelle du poisson à des proies invertébrées traitées (five tests) et non traitées (five tests). Les proies retenues pour les expériences étaient : *Daphnia pulex, D. magna* (Cladocères), *Hyalella azteca* (Amphipodes), et deux tailles d'*Enallagma* sp. (Zygoptères). Le cuivre n'avait aucun effet sur la distance de réaction du poisson au zooplancton. On a observé une relation négative dose-réponse significative pour les taux de consommation de tous les groupes de proies non traitées mais non de proies traitées. La durée de manipulation des proies chez les crapets arlequins capturant des proies traitées ou non augmentait nettement en fonction de la concentration de cuivre et constituait le paramètre le plus régulièrement sensible parmi les paramètres mesurées. L'efficacité de la capture chez le crapet arlequin, bien que modifiée par le cuivre pour certains types de proies, n'était pas une mesure aussi constante du stress toxique. Cette étude indique que des mesures d'ordre mécanique constituent des indicateurs précieux des effets des produits toxiques sur le comportement alimentaire des poissons et que des concentrations de cuivre proches des critères établis par l'US Environmental Protection Agency pour la qualité de l'eau (18 à 28 $\mu g \cdot L^{-1}$) peuvent modifier la consommation de nourriture et réduire la croissance des poissons dans le milieu naturel.

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Establishment of water quality criteria and assessment of a chemical's potential hazard to aquatic life rely primarily on standard acute and chronic toxicity tests (USEPA 1985a; Maki 1979). Though these tests are often sensitive to xenobiotic effects on the physiological components of fish reproduction, growth, and survival, they do not assess sublethal alterations in ecologically important behaviors. Behavioral tests generally have been excluded from the hazard assessment process primarily because of the lack of test standardization and the difficulty of field validation of laboratory results. These drawbacks seem to be rectifiable with further research. Several reviews have demonstrated the sensitivity of fish behavioral tests to toxicant stress (Olla et al. 1980; Westlake 1984; Little et al. 1985; Rand 1985; Atchison et al. 1987).

Standard chronic toxicity tests may be insensitive to some effects. For example, Woltering (1984) questioned the utility of growth measures in determining the lowest observed effect concentration (LOEC) because this parameter was less sensitive than reproduction or survival in more than 70% of the studies in the literature. However, the relative insensitivity of growth as an indicator of toxicant stress may be attributed to the manner in which growth and feeding studies are conducted. Fish are typically fed a high density and low variety of nonliving food (e.g., trout pellets, dried flakes, frozen brine shrimp) in an unstructured environment. Consequently, fish are not challenged to locate, pursue, and capture specific prey as they would

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be under natural conditions. The behavioral aspects of growth are seldom considered in these laboratory tests, yet may be the most important components of toxicant effects in the field.

In previous studies, the most common observation of toxicant-altered feeding behavior was cessation of feeding or reduction in the amount of artificial food consumed (Sandheinrich and Atchison 1990). Changes in the number of live prey attacked and captured, latency to feed, and capture efficiency have also been documented. In tests using live prey, treated fish were typically presented with untreated prey and allowed to feed for a predetermined time. Significant differences in prey consumption, as compared with control fish, were used as an indication of a chemical effect. These tests did not compare the consistency of effect with treated prey or with different prey types. These previous studies had little basis in ecological theory, and their strictly empirical nature precluded development of testable hypotheses amenable to field verification of laboratory results. Several studies (e.g., Werner 1977; Mittelbach 1981; Wright and O'Brien 1984) have demonstrated the utility of a mechanistic approach in using laboratory data to predict the diet and habitat use of fish in the field. It remains now to demonstrate the utility of this approach in toxicological studies involving fish behavior.

We chose copper for this study because of its association with many point and nonpoint industrial sources and the application of copper sulfate as an algicide and molluscicide. More behavioral toxicological research has been done on copper than on any other metal (Atchison et al. 1987), and much information is available on its toxicity to aquatic life and on its chemistry (e.g., Lett et al. 1976; Nriagu 1979; Anderson et al. 1980; Ingersoll and Winner 1982).

Our objectives were (1) to compare copper concentrations that alter feeding behavior of bluegill (*Lepomis macrochirus*) with those estimated to be below concentrations that change growth, reproduction, and survival of this species in the laboratory; (2) to compare copper concentrations causing altered feeding behavior in bluegills with concentrations designated by the U.S. Environmental Protection Agency (USEPA 1985b) as safe for aquatic life; (3) to compare the sensitivity of copper-induced changes in mechanistic measures of foraging (reaction distance, handling time) to more common measures of foraging success (number of prey captured and capture efficiency); and (4) to assess these behavioral changes by using both untreated and treated prey of various species and sizes.

Methods

Test Organisms

Bluegill used in behavioral tests were obtained from a private hatchery. Damselfly naiads, *Enallagma* sp. (Zygoptera: Coenagrionidae) were collected from local ponds. Amphipods (*Hyalella azteca*) and zooplankton (*Daphnia magna* and *D. pulex*) were from laboratory cultures. All test organisms were cultured or maintained in water with the same chemical characteristics (mean ± SE; $n = 12$) as water used in the experiments (temperature, 21.5 ± 1.9°C; dissolved oxygen, 8.5 ± 0.4 mg L^{-1}; pH, 7.5 ± 0.4; alkalinity, 31.4 ± 6.6 mg L^{-1} as $CaCO_3$; hardness, 157 ± 9 mg L^{-1} as $CaCO_3$). Bluegill were acclimated in the lab for at least 2 wk before a test.

Functional Response Experiment

Ten functional response tests investigated the effects of copper on the ability of bluegill to capture different types of prey which had been either treated (five tests) or untreated (five tests) (Table 1). Each test with untreated prey used 48 bluegill, six assigned randomly to each of eight 35-L flow-through tanks. Individual bluegill were identified by cold branding (Everest and Edmundson 1967; Fugihara and Nakatani 1967). Fish were maintained at 22°C under a 16-h light: 8-h dark photoperiod, and were fed frozen brine shrimp (*Artemia salina*) until satiation twice daily during a 1-wk acclimation period. Fish were fed *ad libitum* immediately after each day's foraging trial and then starved for approximately 18 h before the start of a new trial the next day.

Each test consisted of a control period (4–6 d) followed by a treatment period (4 d). The experimental format included daily feeding trials which involved transferring individual bluegill from their treatment tank to a 60-L aquarium with known prey composition (type, size, density) (Table 1). Aquaria used with zooplankton prey were unmodified. Aquaria used with amphipod and damselfly prey were modified to represent the littoral zone of a lake. Pieces of green polypropylene rope (15 cm long) were anchored along 3/4 of the bottom as artificial aquatic plants (20 m^{-2}). In addition to the artificial vegetation, a 3-cm layer of sand was used in aquaria with *Hyalella*.

Untreated prey were initially introduced into the test aquaria the day before initiation of a test and appeared to rapidly acclimate. Consumed prey were replaced prior to the start of each new feeding trial. Two bluegill from each treatment tank were placed singly in each prey density (eight tanks of fish, six fish per tank, three prey densities). After initiation of feeding, bluegill were allowed to feed for 30 s in zooplankton tests and for 3 min in macroinvertebrate tests and then returned to their respective treatment tanks. If a fish did not feed or search for prey within 5 min, the trial was ended. The order in which the tanks of fish were tested was randomized daily, and individual fish were presented with the same prey density during the entire test.

Data recorded were number of prey attacked, number of prey captured, handling time per prey, capture efficiency, and whether the fish searched for prey or remained motionless at the bottom of the tank (motivation to feed). Capture efficiency was defined as the proportion of prey attacked that were captured. Fish were observed directly; a video camera was also mounted over each tank to record the feeding sequence.

Handling times for *Hyalella and Enallagma* were measured as the time from prey capture until search was reinitiated. Previous studies have found *Daphnia* handling times to be too short to be measured in this manner (Ware 1972; Werner 1977; Mittelbach 1981). Instead, such studies used the asymptotic value of time per prey captured as prey density increased to determine minimum handling time. In this study, however, the bluegill did not feed continuously during the 30-s time interval, especially those treated with copper. Therefore, average handling times for bluegill continuously feeding over shorter times were measured from the video tapes. Time required to capture three or more *Daphnia* was measured and divided by the number of prey captured − 1. After the control feeding period, six bluegill in each of the eight tanks were exposed to one of four copper concentrations (two replicates per concentration). Measured mean copper concentrations (± SE; $n = 12$) were 5 ± 5, 31 ± 12, 180 ± 60, and 1710 ± 136 μg L^{-1}. Respectively, these concentrations represented: (1) a control, (2) the proposed 1984 USEPA water quality criteria for copper (based on water hardness of 165 mg L^{-1} $CaCO_3$) expressed as a 30-d maximum (USEPA 1984), (3) the approximate maximum acceptable tox-

TABLE 1. Predator (standard length), prey (length), prey density, and copper concentrations used in functional response and reaction distance experiments.

Predator (mm ± SD)	Prey (mm ± SD)	Prey density (Number L^{-1})	Copper Concentrations ($\mu g\ L^{-1}$)
	Functional Response — Untreated Prey		
bluegill (47.7 ± 2.9)	*Enallagma* sp. (13.6 ± 1.0)	0.5, 2.6, 5.3	5, 31, 180, 1710
bluegill (48.4 ± 2.1)	*Enallagma* sp. (8.2 ± 0.6)	2.6, 5.3, 10.6	5, 31, 180, 1710
bluegill (39.8 ± 2.3)	*Hyalella azteca* (3.2 ± 0.9)	3, 6, 18	5, 31, 180, 1710
bluegill (43.5 ± 3.9)	*Daphnia magna* (2.4 ± 0.3)	0.5, 2.5, 15	5, 31, 180, 1710
bluegill (51.8 ± 2.9)	*Daphnia pulex* (1.6 ± 0.3)	2.5, 15, 30	5, 31, 180, 1710
	Functional Response — Treated Prey		
bluegill (46.2 ± 3.5)	*Enallagma* sp. (13.6 ± 1.0)	0.5, 2.6, 5.3	5, 31, 180
bluegill (54.7 ± 3.0)	*Enallagma* sp. (9.4 ± 0.9)	2.6, 5.3 10.6	5, 31, 180
bluegill (40.3 ± 2.6)	*Hyalella azteca* (3.2 ± 0.9)	3, 6, 18	5, 31, 180
bluegill (54.2 ± 2.9)	*Daphnia magna* (2.4 ± 0.3)	0.5, 2.5, 15	5, 31
bluegill (49.6 ± 2.4)	*Daphnia pulex* (1.6 ± 0.3)	2.5, 15, 30	5, 31
	Reaction Distance — Untreated Prey		
bluegill (43.3 ± 3.4)	*Daphnia magna* (2.5 ± 0.3)	1[a]	5, 31, 180, 1710
bluegill (51.8 ± 2.9)	*Daphnia pulex* (1.6 ± 0.3)	1[a]	5, 31, 180, 1710

[a]Only one prey was presented at a time to bluegill in reaction distance trials.

icant concentration (MATC) for bluegill exposed to copper, and (4) approximately one-half of the 96-h LC50 for bluegill in the laboratory dilution water (Sandheinrich 1988). Subsequent to the initiation of this study, final ambient water quality criteria for copper were published (USEPA 1985b). For water with hardness of 165 mg L^{-1} $CaCO_3$, the criteria state that freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-d average and 1-h average copper concentration does not exceed 18 and 28 $\mu g\ L^{-1}$, respectively, more than once every 3 years on the average. A MATC of 150 $\mu g\ L^{-1}$ was calculated by using the application factor of 0.04 derived from a previous chronic study (Benoit 1975) and a 96-h LC50 of 3750 $\mu g\ L^{-1}$ determined by Sandheinrich (1988). Reagent grade anhydrous copper sulfate was mixed with distilled water, acidified with 5 mL HC1 per litre of stock solution, and distributed via a peristaltic pump and a dilution water system as a constant flow to the treatment tanks. Stock solutions for control tanks were made from distilled water and acid only. Each tank received aerated, dechlorinated, charcoal-filtered municipal tap water at a rate of 100 ± 5 mL per minute for a calculated replacement time of 4 h.

After the first 24 h of copper exposure, the feeding behavior of each fish was again tested during the next 4 d of exposure. During the feeding trials, the fish were removed from their treatment tanks and presented with untreated prey in test tanks with water containing only background levels of copper.

Feeding tests conducted with fish and prey exposed to copper had control periods similar to those previously described. Copper concentrations used during the treatment period were at or below the 48-h LC50 for the invertebrate species used in each experiment (Sandheinrich 1988). There was a pair of control tanks for each pair of fish tanks receiving copper. For example, in experiments using treated *Enallagma* as prey, two tanks received 31 $\mu g\ L^{-1}$ copper and two tanks served as controls for fish exposed to 31 $\mu g\ L^{-1}$ copper. Fish in these four tanks were presented with prey that had been treated with 31 $\mu g\ L^{-1}$ copper. In addition, two tanks received 180 $\mu g\ L^{-1}$ copper and two tanks served as controls for fish exposed to 180 $\mu g\ L^{-1}$ copper. All fish in these four tanks were presented with prey treated with 180 $\mu g\ L^{-1}$ copper. During the treatment period, invertebrates were maintained in aquaria that received overflow water from tanks in which fish were exposed to copper. There were no untreated prey. Copper-exposed fish, as well as controls, were presented with treated prey in test tanks with copper concentrations similar to that of the fish exposure. Treatment periods were often less than four days because of mortality of copper-treated invertebrates and reductions in stock prey populations. More than 1900 functional response trials were done using these methods.

Reaction Distance Experiment

An experimental design similar to that described for the functional response experiment with untreated prey was used to assess copper effects on the ability of bluegill to locate prey. In two separate tests, copper effects on the reaction distance of bluegill to one size class of untreated *Daphnia magna* and one size class of untreated *D. pulex* were determined (Table 1).

Reaction distance was defined as the distance between the fish and prey at which the fish oriented toward and began pursuit of the prey.

Three fish were each assigned to the eight treatment tanks. Reaction distance of each bluegill was determined for 4 consecutive days before and during copper exposure (5, 31, 180, 1710 μg L^{-1}) by using methods similar to those described by Confer and Blades 1975; Eggers 1977; and O'Brien 1979. Individual bluegill were transferred to a clear plastic chamber (100 × 10 × 10 cm) with a ruled background and presented with single prey via a manually controlled device that dropped individual *Daphnia* into the chamber at the opposite end from the fish and beyond its visual range. Fish were allowed to feed for 10 min or until 10 *Daphnia* were consumed. After each trial, the fish were returned to their respective tanks. A video cassette recording system was used to record each trial for later measurement of reaction distance. More than 190 reaction distance trials were done.

Water Chemistry

Copper was analysed twice for each test. Water samples were taken from each tank, acidified to pH <2 with HCl and refrigerated for later analysis. Samples containing nominal copper concentrations of > 1000 μg L^{-1} were analyzed by direct aspiration into an atomic absorption-flame ionization spectrophotometer. Samples containing nominal concentrations less than 1000 μg L^{-1} copper were first extracted with an organic solvent based on techniques of Brown et al. (1970) and Kinrade and VanLoon (1974) as modified by Shephard (1976) and then analyzed by atomic-absorption flame-ionization spectrophotometry.

Statistical Procedures

Functional response experiments were a split-plot statistical design with repeated measures in which the treatment tanks were the experimental units (Winder 1971). The method allows the use of data from several dates when comparing treatment effects, but without the assumption that sampling dates are independent of each other. The linear statistical model contained copper as the main-plot treatment effect. In testing this effect for experiments with untreated prey, the variance of the treatment tanks nested within copper was used as the error term (F with 1,4 df). The subplot contained the effects of prey density (F with 2,32 df) and interactions of copper with prey density (F with 6,32 df). The variance of fish nested within copper, tank, and prey density plus the variance of the interaction between density and tank nested within copper was used as the subplot error term. A similar analysis of variance was done for experiments with treated prey, but with slightly different degrees of freedom because of fewer copper concentrations used than in experiments with untreated prey. Similarly, copper effects of bluegill reaction distance (F with 1,4 df) were analyzed by using the variance of tanks nested within copper as the error term. All analysis of variance (ANOVA) tests were done by using the Statistical Analysis System (SAS) General Linear Models (GLM) procedure (SAS Institute, Inc. 1985). Null hypotheses were rejected at $P < 0.05$.

Results

The number of untreated prey captured by copper-treated and untreated bluegill generally increased ($P < 0.05$) with prey density (Fig. 1). With few exceptions (e.g., large damselflies at 0.5 prey L^{-1}), control fish consumed more prey than treated fish. Copper had no significant effect on the increased feeding response of bluegills as prey density increased, except for experiments using untreated small damselflies and *D. magna* ($P < 0.05$).

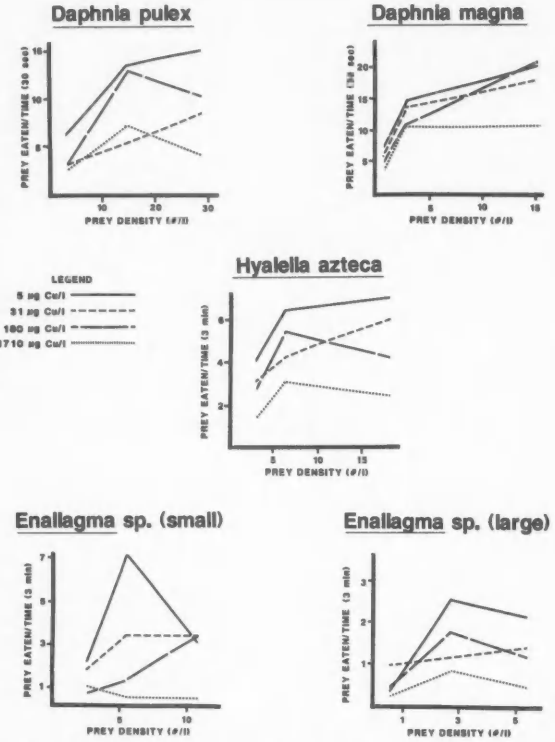

FIG. 1. Bluegill foraging response to five types of untreated prey following exposure to copper. Prey consumption rates of control and treated bluegill are shown at various prey densities. Shown in the legend are copper concentrations to which fish were exposed.

Fish exposed to copper captured fewer prey than controls ($P < 0.05$). When averaged over untreated prey density, a significant negative relationship between prey consumption and copper concentration was observed (Fig. 2). Bluegill exposed to copper concentrations as low as 31 μg L^{-1} consumed 27.2% ± 16.9% ($n = 5$) fewer prey than untreated fish. Deviations from log linear models were not significant.

The number of copper-treated prey captured by treated and untreated fish also increased with prey density ($P < 0.05$) (Fig. 3), with the exception of the tests using treated *D. pulex* and large damselflies. Except for the test with large damselflies, copper had no significant effect on the increased feeding response of bluegills as treated prey density increased. The rate of capture of prey treated with 31 μg L^{-1} copper by unexposed fish was similar to that of prey treated with 180 μg L^{-1} copper. The apparent negative relationship between consumption of treated prey and copper concentration was not statistically significant (Fig. 4).

The amount of time required for copper-exposed bluegill to handle individual, untreated prey significantly increased with copper concentration ($P < 0.05$) except for *D. pulex* (Fig. 5). Handling times were not affected by prey density, and devia-

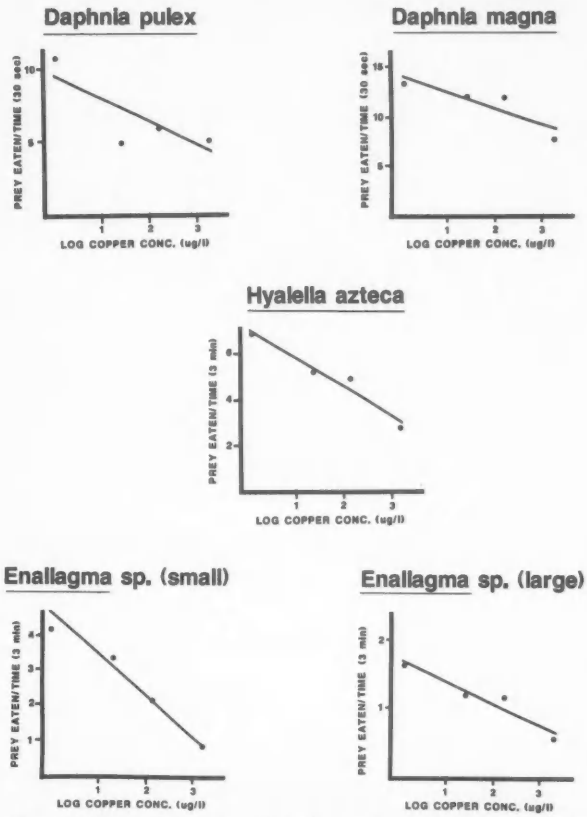

FIG. 2. Effects of copper on bluegill consumption of five types of untreated prey. Consumption rates were averaged over all prey densities. The following values resulted from exposure concentration: *Daphnia pulex*: $R^2 = 0.50$, $P < 0.05$, SE = 1.58. *Daphnia magna*: $R^2 = 0.70$, $P < 0.05$, SE = 0.87. *Hyalella azteca*: $R^2 = 0.85$, $P < 0.01$, SE = 0.49. *Enallagma* sp. (small): $R^2 = 0.88$, $P < 0.01$, SE = 0.81. *Enallagma* sp. (large): $R^2 = 0.84$, $P < 0.01$, SE = 0.13.

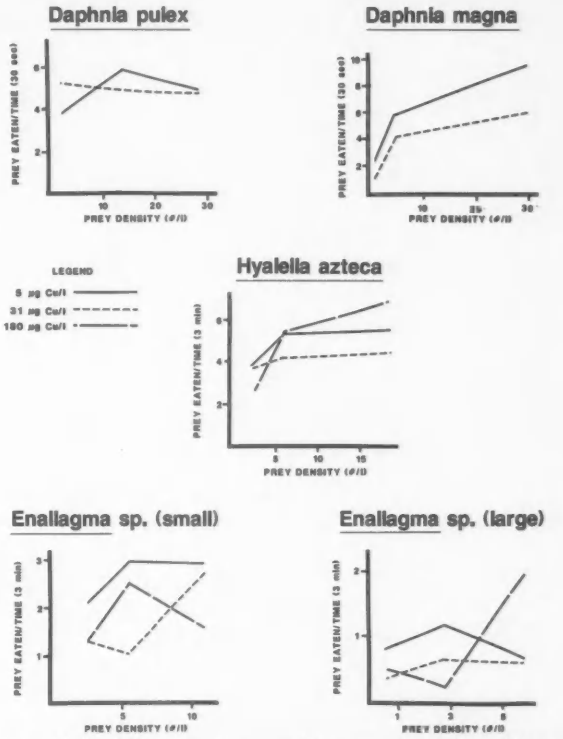

FIG. 3. Bluegill foraging response to five types of treated prey following exposure to copper. Prey consumption rates of control and treated bluegill are shown at various prey densities. Shown in the legend are copper concentrations to which fish and prey were exposed.

tions from the log linear model were nonsignificant. Handling times for treated prey followed the same pattern (Fig. 6). Copper-exposed bluegill required a significantly longer handling time than unexposed bluegill for all treated prey ($P < 0.05$), except when *D. pulex* and large *Enallagma* sp. were used. Mortality of treated *D. magna*, induced by the copper exposure, precluded determination of handling times of this prey by bluegill.

Increases in handling times with copper concentration were not consistent for each type of unexposed prey. Figure 7 shows the percentage increase, relative to control, in handling time for copper-treated fish, as a function of relative prey size (prey length/fish length). There was an inverse relationship between increases in handling time and prey size. Bluegill exposed to 31 and 180 μg L^{-1} copper exhibited greater increases in handling times for small prey (e.g., *D. magna*) than for large prey (e.g., large damselflies).

Differences in the proportion of attacked prey that were captured by treated and untreated fish were significant only for bluegill feeding on untreated amphipods, small damselflies, and large damselflies ($P < 0.05$) (Fig. 8). Control fish captured a greater percentage of prey that they attacked than did treated fish. Prey that were attacked but not consumed either escaped or were expectorated by the bluegill.

Copper had no effect on the proportion of fish that searched for prey in the test tanks. Fifty to 100% of fish in each copper concentration searched for prey within 5 min of being put into the test tanks with different densities of prey.

Of all the behaviors measured in the functional response experiments, handling time was most consistently sensitive to copper stress (Table 2). Except for experiments with *Daphnia pulex* and treated large *Enallagma* sp., the handling time of fish exposed to copper was significantly greater than that of controls for both treated and untreated prey. Other parameters, such as prey consumption rates and capture efficiency, were also affected by copper but were not as consistent.

Copper had no effect on the ability of bluegill to visually locate prey. The average reaction distance of treated and untreated bluegill to *D. pulex* and *D. magna* was 8.8 ± 0.6 cm ($n = 4$) and 22.4 ± 0.9 cm ($n = 4$), respectively.

Discussion

Standard chronic toxicity studies test chemical effects primarily on physiological aspects of growth, but generally not on behavioral aspects that could also be significantly affected. Benoit (1975) reported that adult bluegill growth, reproduction, and survival were reduced by copper concentrations as low as 162 μg L^{-1} (water hardness, 45 mg L^{-1} $CaCO_3$). Larval bluegill survival after 90 days exposure to copper was adversely

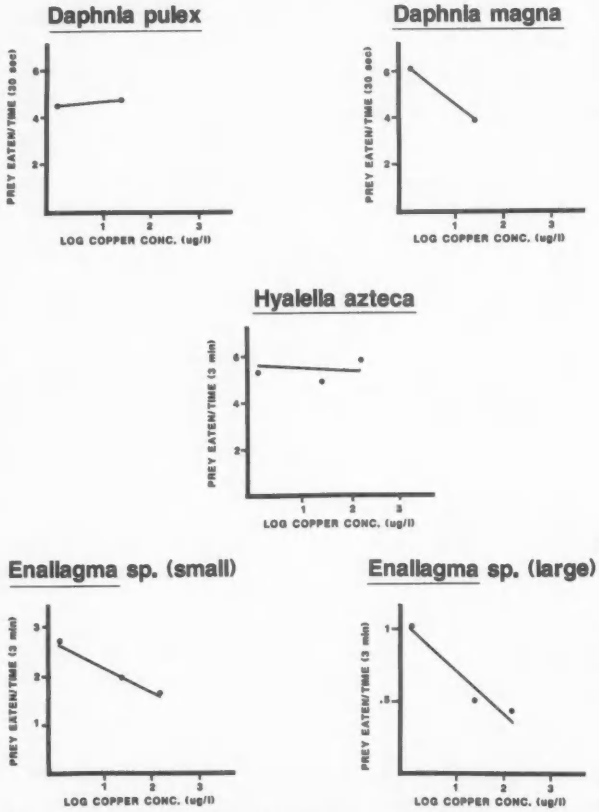

FIG. 4. Effects of copper on bluegill consumption of five types of treated prey. Consumption rates were averaged over all prey densities. Prey and fish were exposed to similar copper concentrations. The following values resulted from regression analysis and are shown relative to exposure concentration: *Daphnia pulex:* $R^2 = 0.05$, $P > 0.50$, SE = 0.35. *Daphnia magna*: $R^2 = 0.44$, $P > 0.25$, SE = 1.21. *Hyalella azteca*: $R^2 = 0.009$, $P > 0.50$, SE = 0.53. *Enallagma* sp. (small): $R^2 = 0.37$, $P > 0.05$, SE = 0.37. *Enallagma* sp. (large): $R^2 = 0.36$, $P > 0.05$, SE = 0.29.

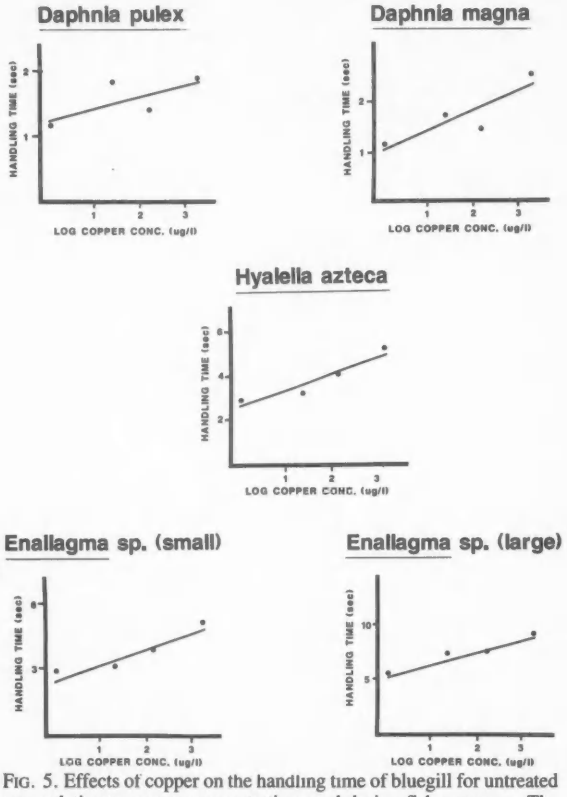

FIG. 5. Effects of copper on the handling time of bluegill for untreated prey relative to copper concentration used during fish exposure. The following values resulted from regression analysis: *Daphnia pulex*: $R^2 = 0.37$, $P > 0.05$, SE = 0.16. *Daphnia magna:* $R^2 = 0.63$, $P < 0.05$, SE = 0.20. *Hyalella azteca:* $R^2 = 0.63$, $P < 0.05$, SE = 0.59. *Enallagma* sp. (small): $R^2 = 0.68$, $P < 0.05$, SE = 0.43. *Enallagma* sp. (large): $R^2 = 0.79$, $P < 0.05$, SE = 0.53.

affected at 40–162 μg L^{-1}. On the basis of this information, Benoit (1975) determined a maximum acceptable toxicant concentration (MATC) of between 21–40 μg Cu L^{-1}. In this study, copper altered bluegill feeding behavior at concentrations less than our calculated MATC of 150 μg L^{-1}. Copper concentrations as low as 31 μg L^{-1} reduced consumption and increased handling times of bluegill foraging on a variety of prey types. Although a variety of chemical processes control the toxicity of copper in freshwater ecosystems (Sprague 1985), this study suggests that bluegill growth and prey choice in natural systems may be altered by copper concentrations near the federal water quality criteria for the protection of aquatic life chronically exposed to copper (USEPA 1985b).

Copper had no affect on the ability of bluegill to locate prey as tested in the reaction distance experiments. Copper has been reported to damage corneas of larval striped bass, *Morone saxatilis* (Bodammer 1985). But the most common report of tissue damage caused by copper is to gills and some sensory tissues such as the olfactory and lateral line systems (Baker 1969; Gardner and LaRoche 1973). However, other chemicals may cause diminished vision in fish, so reaction distance should not be ruled out as an effective tool on the basis of this copper study alone. Nyman (1981) reported that lead reduced the reaction distance of zebrafish, *Brachydanio rerio*, feeding on *Daphnia*.

The capture efficiency (no. of prey captured/no. of prey attacked) was not a consistently sensitive measure of sublethal copper effects. Previous studies have noted reduced capture efficiency of bluegill exposed to fluorene when preying on untreated chironomid larvae (Finger et al. 1985), and also of largemouth bass (*Micropterus salmoides*) exposed to pentachlorophenol when preying on poeciliids (Mathers et al. 1985) and brine shrimp (Brown et al. 1987). In this study, significant differences in capture efficiency between control and copper-treated bluegill occurred in only three of five experiments using untreated prey and in none of the experiments using treated prey. Notably, differences in capture efficiency occurred with large prey, suggesting that the sensitivity of this metric may be related to the ratio of predator size to prey size.

Reductions in prey consumption or cessation of feeding is the most common observation of toxicant-altered feeding behavior in the literature (Sandheinrich and Atchison 1990). In this study, reductions in prey consumption by copper-treated bluegill were significant only for fish feeding on untreated prey.

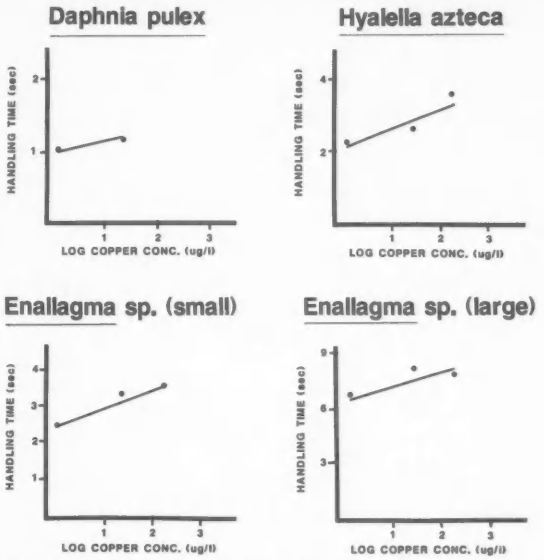

FIG. 6. Effects of copper on the handling time of bluegill for treated prey relative to copper concentration used during fish and prey exposure. The following values resulted from regression analysis: *Daphnia pulex*: $R^2 = 0.38$, $P > 0.25$, SE = 0.12. *Hyalella azteca*: $R^2 = 0.83$, $P < 0.01$, SE = 0.10. *Enallagma* sp. (small): $R^2 = 0.80$, $P < 0.01$, SE = 0.19. *Enallagma* sp. (large): $R^2 = 0.35$, $P > 0.05$. SE = 0.50.

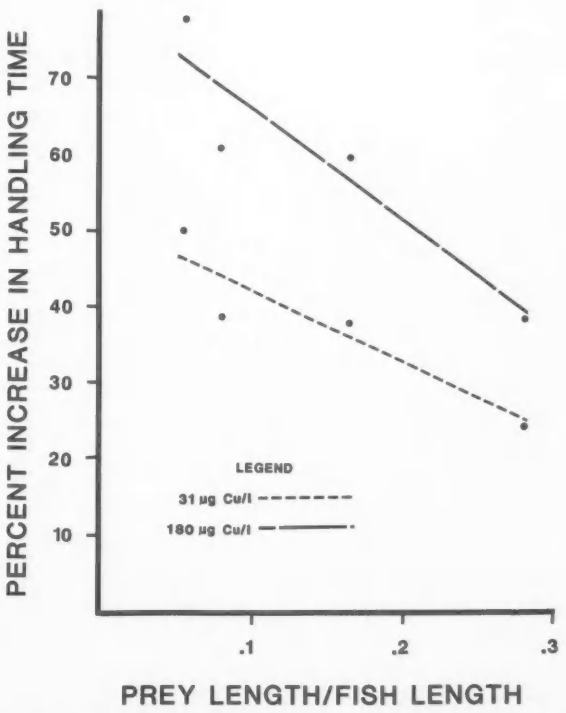

FIG. 7. Percentage increase, relative to controls, in handling time of bluegill treated with copper as a function of relative size of untreated prey (prey length/fish length).

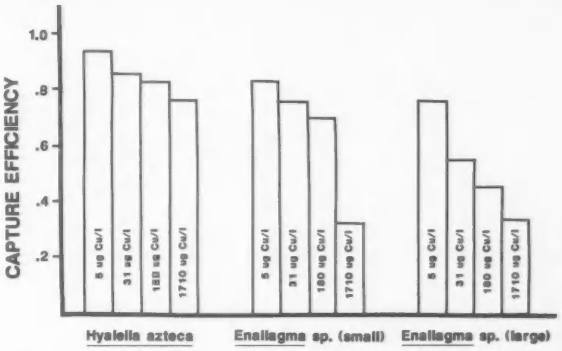

FIG. 8. Capture efficiency (no. captured/no attacked) of control and treated bluegill preying on untreated amphipods (*Hyalella azteca*) and two sizes of untreated damselfly naiads (*Enallagma* sp.).

TABLE 2. Summary of copper effects on bluegill foraging behavior. Differences between copper-treated and control fish significant at $P<0.05$ (**) or not significant (NS).

Prey	Handling time	Prey capture	Capture efficiency	Motivation to feed
		Untreated Prey		
Enallagma (large)	**	**	**	NS
Enallagma (small)	**	**	**	NS
Hyalella azteca	**	**	**	NS
Daphnia magna	**	**	NS	NS
Daphnia pulex	NS	**	NS	NS
		Treated Prey		
Enallagma (large)	NS	NS	NS	NS
Enallagma (small)	**	NS	NS	NS
Hyalella azteca	**	NS	NS	NS
Daphnia magna	—	NS	NS	NS
Daphnia pulex	NS	NS	NS	NS

Change in handling time was the most consistent measure of altered feeding behavior for copper-treated bluegill feeding on both untreated and treated prey. Copper increased handling times, relative to controls, of treated fish for all prey except in the two experiments using *D. pulex* and the experiment using treated large *Enallagma* sp. Handling time per prey is a function of relative prey size (prey length/fish length), which increases exponentially above a minimum ratio of prey length/predator length and remains relatively constant below this critical minimum (Schoener 1969; Mittelbach 1981). The ratio of *D. pulex* length to bluegill length used in this study was 0.031. This was less than the critical minimum of 0.034 determined by Mittelbach (1981) below which the handling time remains constant at approximately 1 s. Increases in handling times for bluegill on *D. pulex* caused by copper may not have been sufficiently large to exceed the minimum constant time.

Previous studies also have noted toxicant-induced increases in prey handling time. Nyman (1981) reported that lead increased handling time of zebrafish feeding on *Daphnia*. Cairns and Loos (1967) found that zebrafish exposed to zinc, alkyl benzene sulfonate, or potassium dichromate required more time than control fish to consume tubifex worms. The 1-mm sections of tubifex were often regurgitated repeatedly after capture before they were finally retained.

Handling time is an important component of several models predicting the diet of bluegill in the wild (Werner and Hall 1974;

Mittelbach 1981). In this study, percentage increases in handling time caused by copper were not constant with respect to prey size. Laboratory data suggest that copper will increase the cost of capturing prey, with the increase in cost being proportionally greater for small than for large prey. This would not only result in a decrease in the net energy gain of a fish, but it would also be expected to increase the average size of prey consumed and alter the size-frequency distribution of prey in the gut of copper-treated fish in the wild.

In summary, sublethal copper concentrations decreased prey consumption and increased handling times of bluegill preying on five different invertebrate prey. Copper had no effect on the reaction distance of bluegill to two sizes of zooplankton. Changes in handling time were more consistent measures of copper stress than prey consumption, capture efficiency, or the proportion of fish searching for prey. This suggests that mechanistic measures may be sensitive indicators of changes in diet and net energy intake of fish caused by toxicant stress. In addition, current foraging models can make use of these mechanistic measures to predict fish diet in the wild. This approach should allow development of testable hypotheses of predator–prey interactions before field validation of laboratory results.

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Bivariate Normal Swimbladder Size Allometry Models and Allometric Exponents for 38 Mesopelagic Swimbladdered Fish Species Commonly Found in the North Sargasso Sea

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Saenger, R. A. 1989. Bivariate normal swimbladder size allometry models and allometric exponents for 38 mesopelagic swimbladdered fish species commonly found in the North Sargasso Sea. Can. J. Fish. Aquat. Sci. 46: 1986-2002.

With x and y log-transformed fish standard length l and equivalent swimbladder radius R, respectively, probability density distributions $p(x,y)$ for mesopelagic swimbladdered fish species are assumed to be bivariate normal. From archival measurements, models are developed for 36 nonregressive species, 2 regressive species, and selected species groups found off Bermuda. Statistical tests suggest the hypothesis is valid for many of these species, and that failures are due largely to small sample variability and bias in the available data. Marked differences between regression, major axis, and reduced major axis allometric exponents, which sometimes occur when x,y correlation is low, are explained. A dimensionless allometric law $R'_* = k_* (l'_*)^m$ involving structural variables l'_* and R'_* scaled to l_{max} is used to compare specific swimbladder growth trends. Major axis estimates of m_* and k_* for the nonregressive species group are each lognormally distributed with respective means $\tilde{m} = 3/2$ and $\tilde{k}_* = 0.04$. A structural relation model for this group based on the hypothesis that swimbladder wall area grows approximately as the first power of fish volume or mass is verified. Last, detailed buoyancy properties are calculated for 6 nonregressive species with bivariate normal (x,w_*)-distributions, where w_* is the logarithm of percentage swimbladder volume.

Quand on exprime sous forme logarithmique la longueur standard des poissons, l, et le rayon équivalent de la vessie natatoire, R, suivant l'axe des x et des y respectivement, les densités de probabilité $p(x,y)$ pour les espèces de poissons pélagiques ayant une vessie natatoire devraient suivre un loi normale à deux variables. Des modèles s'appliquant à 36 espèces non régressives, à deux espèces régressives et à des groupes d'espèces sélectionnées provenant du large des côtes des Bermudes ont été élaborés à partir de mesures d'archives. Des tests statistiques ont laissé entendre que l'hypothèse de départ était valable pour plusieurs de ces espèces et que les résultats non conformes à cette hypothèse sont dus en grande partie à la variabilité inhérente au fait que nos échantillons étaient de petite taille et à certains biais dans les données disponibles. Les différences marquées observées en ce qui a trait à la régression, à l'axe principal, et aux exposants allométriques réduits de l'axe principal, différences qui apparaissent parfois quand la corrélation entre x et y est faible, sont expliquées. Une loi allométrique non dimensionnelle, $R'_* = k_*(l'_*))^m$, faisant appel aux variables structurales l'_* et R'_* réduites suivant l_{max} a été utilisée pour comparer les tendances spécifiques en ce qui a trait à la croissance de la vessie natatoire. Les estimations suivant l'axe principal de m et k_*, dans le cas des espèces non régressives, suivant chacune une distribution lognormale dont les moyennes respectives sont : $\tilde{m} = 3/2$ et $\tilde{k}_* = 0{,}04$. Nous avons aussi testé un modèle structural de relations relatif à ce groupe d'espèces et fondé sur l'hypothèse que la surface de la paroi de la vessie natatoire croît approximativement suivant la première puissance du volume ou de la masse du poisson. Enfin, nous avons calculé de façon détaillée les caractéristiques de flottabilité de six espèces non régressives qui suivent une loi normale à deux variables (x,w_*), où w_* est le logarithme du volume de la vessie natatoire exprimé en pourcentage.

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The gas-filled swimbladder is known to be an important contributor to the buoyancy of many species of mesopelagic swimbladdered fish (Marshall 1960). It is also responsible for the large acoustic backscatter cross sections of these fish below 20 kHz which make them prime contributors to low frequency volume reverberation in the ocean (Farquhar 1971). Physical analysis of the role of the swimbladder in buoyancy control and acoustic scattering requires swimbladder size allometry models which describe both the trend of swimbladder growth with increasing fish size and the often pronounced biological variability associated with the trend. It is our purpose here to develop such models for 38 swimbladdered species commonly found at the Ocean Acre site near Bermuda. These species are a subset of the 55 swimbladdered species identified by Brown and Brooks (1974) as the predominant contributors to low frequency volume scattering at Ocean Acre.

Since the classic work of Huxley (1932), the trend of organ growth in a species has usually been characterized with the simple allometric law. Letting l represent fish standard length, R the equivalent spherical radius of the swimbladder, and l_0 unit dimensional length (1 mm here), we write the law as $(R/l_0) = k(l/l_0)^q$ mainly to facilitate its representation in loga-

rithmic form. The quantities R/l_0, k, and l/l_0 are formally dimensionless with numerical values depending on l_0, while the "allometric exponent" q is a dimensionless physical constant in the usual sense. Distinct types of swimbladder growth are associated with different ranges of q. For nonregressive species, distinguished by positive values of q, three types of growth are defined: negative allometric growth ($0 < q < 1$), isometric growth ($q = 1$), and positive allometric growth ($q > 1$). For these three ranges of q, dR/dl is positive, respectively decreasing, remaining constant, or increasing as l increases. For regressive species, distinguished by negative q, bladder regression commences at some stage of fish development, at which point dR/dl becomes negative, decreasing in absolute value with increasing l. Introducing $x = \ln(l/l_0)$ and $y = \ln(R/l_0)$, the allometric law may be expressed in the alternative form $y = a + qx$ with $a = \ln k$.

The linear trend of y with x implied by the allometric law is more or less evident in scattergram plots of x,y data for mature members of the 38 Ocean Acre swimbladdered species. Almost always, large biological variability is present. To mathematically characterize the trend and associated biological variability for a typical swimbladdered species, we shall assume that the random variables x,y are described by a species-specific bivariate normal probability density $p(x,y)$, a model particularly convenient for physical applications.

In the Materials and Methods section, after a description of the data, the bivariate normal model formalism and testing procedures for bivariate normality are briefly described. Theoretical relations between the regression, major axis, and reduced major axis estimators for the allometric exponent q are given in terms of the correlation coefficient and the ratio of the sample standard deviations of x and y. Next, the linear structural model and the problem of choosing an estimator for q are briefly reviewed. A dimensionless allometric law structural relation is then formulated. The section concludes with the derivation of a physical model for the structural relations expected for nonregressive swimbladdered species.

Bivariate normal models for 38 Ocean Acre species and effective models for certain species groups are presented in the Results and Discussion section, and the reasonableness of the bivariate normal hypothesis for individual species is examined with the help of a set of statistical tests. Regression, major axis, and reduced major axis estimates of the allometric exponent for the 38 species are then discussed. Next, comparisons of swimbladder growth trends in the 36 nonregressive species are made on the basis of their dimensionless allometric law structural relations. Finally, the buoyancy implications of swimbladder size and its variability in six of the nonregressive species are investigated.

Materials and Methods

Swimbladder Allometry Data

Between 1967 and 1972 the Smithsonian Institution, the University of Rhode Island and the Naval Underwater Systems Center performed a series of biological sampling and acoustic backscatter experiments at Ocean Acre, a 1-degree quadrangle southeast of Bermuda (Gibbs et al. 1971 a,b; Brown and Brooks 1974). A limited series of similar experiments was performed in the Mediterranean during August–October, 1970 (Goodyear et al. 1972). Fifty-five mesopelagic swimbladdered fish species, some of which were also caught in the Mediterranean, were identified as the predominant contributors to volume scattering at Ocean Acre (Brown and Brooks 1974). During the mid-1970's, swimbladder size allometry data for these species were obtained from the Smithsonian Institution and the University of Rhode Island for analysis (Brooks 1976, 1977) and bioacoustic modeling (Brooks and Brown 1977) at the Naval Underwater Systems Center. Data for 44 of the 55 bladdered species were derived from specimens in the Ocean Acre collections, while data for the 11 other species were derived from specimens in the Mediterranean collections. Much has been written on the vertical distribution, ecology, and swimbladder characteristics of these species — see, e.g., Keene 1970; Gibbs et al. 1971b; Goodyear et al. 1972; Bond 1974; Kleckner 1974; Howell 1975; Karnella 1983; and Gibbs and Krueger 1987.

On retrieval the specimens were preserved in 10% formalin (4% formaldehyde solution). Later, in the laboratory, they were washed and transferred through a series of ethanol solutions of increasing concentrations up to 70%. Specimens covering the size range of the species were selected for dissection under a low-power microscope. The allometric data for each specimen consists of a measurement of standard length l with dial calipers and measurements with an ocular micrometer of the anterior — posterior length $2a$ and maximum width $2b$ of the external swimbladder wall (Goodyear et al. 1972; Kleckner 1974). The swimbladder was taken as a prolate spheroid with volume $V_B = (4\pi/3)ab^2$ (Capen 1967) and the radius R of the equivalent spherical volume was calculated from $R = (ab^2)^{1/3}$. We exclusively consider the size allometry properties of R, since those of a and b are of lesser physical interest here. The positive buoyancy contribution of a gas-filled swimbladder depends on its volume, and hence, R. Also, swimbladder resonance scattering frequencies are simply related to R, with a correction factor depending on b/a which is close to 1 and therefore often neglected in bioacoustic modeling (Andreeva 1964; Love 1978). Information on the allometric properties of a and b may be found in Brooks (1977).

Since shrinkage due to preservation is uncertain, no correction of the original data has been attempted. Thus, plots of the logarithmic variables x,y made from (l,V_B) data of Capen (1967) for *Lampanyctus leucopsarus*, *L. mexicanus*, and *Diaphus theta* show large statistical variability as found here, with no readily discernible systematic differences between data for preserved and fresh specimens. If desired, correction factors for presumed shrinkage of l and R may be introduced into the models given below simply by adding appropriate constants to the corresponding means $\bar{x}$ and $\bar{y}$. Some unknown part of the variability in y found here arises from the use of the prolate spheroid approximation for V_B and the effects of preservative. However, the existence of sizable natural variability in y tends to be supported, e.g., by the large variability in swimbladder gas volume evident in the measurements of Shearer (1971) on fresh specimens of 4 mesopelagic species in which the measurement technique of Kanwisher and Ebeling (1957) was used. Large variability in the swimbladder gas volume of fresh specimens of *Myctophum punctatum* dipnetted at the surface is also seen in the data of Bone (1973) who used a different method of measurement.

Data sets for 38 of the 55 swimbladdered species were considered suitable for modeling. Examination of x,y scattergrams revealed that 36 of the 38 species have nonregressive bladder growth characteristics. Small specimens of nonregressive species, when available, show a rapid initial

TABLE 1. Thirty-eight Ocean Acre swimbladdered species for which bivariate normal models have been developed listed according to family. The number n of data points used and the ranges of standard length l and swimbladder radius R are given for each species. Nonregressive specimens with $R \leq 0.25$ mm are excluded. Data obtained from Mediterranean specimens are indicated by an asterisk. Only the two flagged myctophid species †*Lampanyctus ater* and †*L. cuprarius* have regressive swimbladder growth characteristics.

Species no.	Species	n	l_{min}(mm)	l_{max}(mm)	R_{min}(mm)	R_{max}(mm)
	MYCTOPHIDAE					
1	*Benthosema suborbitale*	11	11.6	26.0	0.27	1.72
2	*Bolinichthys indicus*	31	18.3	39.4	0.42	1.76
5	*Ceratoscopelus warmingi*	37	18.6	66.0	0.28	1.83
7	*Diaphus metapoclampus*	11	24.5	74.6	0.83	5.38
10	**Diaphus rafinesquei*	48	10.6	77.6	0.43	5.75
11	*Diogenichthys atlanticus*	32	11.1	19.4	0.26	0.88
12	**Hygophum benoiti*	65	7.2	44.0	0.26	2.16
13	**Hygophum hygomi*	51	11.7	57.0	0.29	1.94
14	*Hygophum taaningi*	15	12.4	39.8	0.28	1.38
18	*Lampadena urophaos*	35	22.6	78.3	0.33	3.05
19	*Lampanyctus alatus*	11	30.3	51.2	0.66	2.10
20	†*Lampanyctus ater*	35	25.2	97.4	0.18	1.03
21	**Lampanyctus crocodilus*	63	10.5	171.7	0.29	7.77
22	†*Lampanyctus cuprarius*	44	30.3	70.7	0.17	0.42
23	*Lampanyctus festivus*	37	25.3	61.3	0.37	2.39
25	*Lampanyctus photonotus*	43	21.3	64.3	0.50	1.95
26	**Lampanyctus pusillus*	47	13.8	40.0	0.29	1.38
27	*Lepidophanes gaussi*	32	15.4	41.9	0.26	1.91
29	**Lobianchia dofleini*	57	12.0	42.4	0.30	2.00
30	*Lobianchia gemellari*	42	12.8	99.8	0.31	4.51
34	*Notoscopelus resplendens*	60	21.7	72.6	0.38	1.91
35	*Symbolophorus rufinus*	25	20.1	85.9	0.31	3.37
36	*Taaningichthys bathyphilus*	25	17.7	65.9	0.35	2.81
37	*Taaningichthys minimus*	30	22.1	54.2	0.50	2.12
	GONOSTOMATIDAE					
38	*Bonapartia pedaliota*	26	17.0	59.0	0.35	2.74
39	**Ichthyococcus ovatus*	25	12.0	35.0	0.35	1.90
41	*Valenciennellus tripunctulatus*	71	11.2	29.5	0.30	1.39
42	**Vinciguerria attenuata*	41	12.4	36.4	0.27	1.23
43	**Vinciguerria poweriae*	29	13.1	33.7	0.30	1.23
	STERNOPTYCHIDAE					
44	*Argyropelecus aculeatus*	30	9.0	55.9	0.29	2.28
45	**Argyropelecus hemigymnus*	47	7.2	33.8	0.30	1.70
46	*Sternoptyx diaphana*	30	8.9	35.0	0.41	2.09
	MELAMPHAIDAE					
47	*Melamphaes pumilus*	59	10.2	21.4	0.27	1.00
48	*Melamphaes typhlops*	30	9.6	70.8	0.38	3.88
49	*Scopeloberyx opisthopterus*	30	13.0	38.6	0.40	1.71
51	*Poromitra capito*	32	12.0	99.1	0.37	5.21
	ANOPLOGASTERIDAE					
50	*Anoplogaster cornuta*	5	11.5	81.5	0.52	1.46
	OPISTHOPROCTIDAE					
55	*Rhynchohyalus natalensis*	9	16.9	52.5	0.36	1.53

increase of y with x that slows as x increases. For larger specimens, a linear trend of y with x is established that usually persists as x increases. The lower boundary of the linear allometric law region for these nonregressive species is approximately $y = -1.4$, or $R = 0.25$ mm. For the 2 regressive species, data are only available for relatively large specimens in which bladder regression is well underway. The trend of y with x is linear in these data sets also, but the slope is negative. Larger nonregressive specimens and regressive specimens with x,y values lying in a linear allometric law region are termed "mature" for convenience. The number of mature specimens of each species used in modeling is listed in Table 1, along with corresponding ranges of l and R.

Bivariate Normal Modeling Formalism and Statistical Tests

Consider the ensemble of x,y data points for mature specimens of a swimbladdered species obtained by repeated biological sampling of the entire upper water column in an area where the species is commonly found. We assume this x,y population is bivariate normal (Cramér 1955) with unknown theoretical distribution parameters [ξ, η, σ_x, σ_y, ρ]. In practice, only a finite number n of data points will be available, and the thoretical distribution must be approximated by a sample distribution $p(x,y)$ of the form

$$(1)\quad p(x,y) = \frac{1}{2\pi\, s_x\, s_y \sqrt{1 - r^2}}\, e^{-Q(x,y)/2}$$

with

$$(2)\quad Q(x,y) = \frac{1}{1 - r^2}\left[\left(\frac{x - \bar{x}}{s_x}\right)^2 - 2r\left(\frac{x - \bar{x}}{s_x}\right)\left(\frac{y - \bar{y}}{s_y}\right) + \left(\frac{y - \bar{y}}{s_y}\right)^2\right].$$

The parameters $\bar{x}$ and $\bar{y}$ are the sample means of x and y, respectively, s_x^2 and s_y^2 are the corresponding sample variances, and r is the sample correlation coefficient. Maximum likelihood estimators for the respective theoretical parameters [ξ, η, $\sigma^2{}_x$, $\sigma^2{}_y$, ρ] are (Kendall and Stuart 1979)

$$\bar{x} = \frac{1}{n}\sum x,\quad \bar{y} = \frac{1}{n}\sum y$$

$$s_x^2 = \frac{1}{n}\sum (x - \bar{x})^2,\quad s_y^2 = \frac{1}{n}\sum (y - \bar{y})^2$$

$$(3)\quad r = \left[\sum (x - \bar{x})\,(y - \bar{y})\right]/(n\, s_x\, s_y).$$

A parent bivariate normal population with parameters ξ, η, σ_x, σ_y, ρ has many distinctive properties that are reflected by the sample population. Thus, e.g., it has normal x and y marginal probability density functions $N(\xi,\sigma_x^2)$ and $N(\eta,\ \sigma_y^2)$, respectively. Also, the probability density of perpendicular distances from points x,y to the major axis of the theoretical distribution is normal $N(0,\Delta^2)$, with an expected mean-square distance Δ^2 depending on σ_x, σ_y, and ρ^2. Letting $Q'(x,y)$ denote $Q(x,y)$ with $\bar{x}$, $\bar{y}$, ..., r replaced by the corresponding population parameters ξ, η, ..., ρ, the distribution of probability in the x,y plane is such that P% of the distribution lies between the point ξ, η and the ellipse $Q'(x,y) = \chi^2{}_p$, where $\chi^2{}_p$ is the P-fractile of a chi-square distribution with 2 degrees of freedom (Cramér 1946; Hald 1952). By successively setting Q' equal to $\chi^2_{1/J}$, $\chi^2_{2/J}$ ..., where J is any integer larger than 1, a set of concentric homothetic ellipses is defined containing any desired fraction $1/J$ of the distribution between successive curves. The reasonableness of the bivariate normal assumption for our sample distributions is examined below by subjecting the larger sample populations to statistical tests based on these properties.

Allometric Law Structural Relations

Regression estimators for the allometric exponent q — The linear trend in bivariate normal data is usually characterized by some form of mean-square regression line. The familiar y-on-x regression line, commonly used for prediction, is obtained by minimizing the mean-square vertical distance of x,y points from the line. The orthogonal regression line, or major axis, is obtained by minimizing the mean-square perpendicular distance of points from the line. An orthogonal regression line fitted to the standardized variates $(x - \bar{x})/s_x$ and $(y - \bar{y})/s_y$ is a third type of regression line variously termed the reduced major axis (Kermack and Haldane 1950), the geometric mean regression line (Ricker 1973), or the standard major axis (Jolicoeur 1975). The regression coefficients, i.e., the slopes of these lines, represent estimates of the allometric exponent q in Huxley's law $y = a + qx$ under fundamentally different measurement conditions, as discussed in the following section.

The slopes b of the y-on-x regression line, m of the major axis, and g of the reduced major axis are given by

$$(4)\quad b = r(s_y/s_x)$$

$$(5)\quad m = \frac{(s_y^2 - s_x^2) + \sqrt{(s_y^2 - s_x^2)^2 + 4\, s_x^2\, s_y^2\, r^2}}{2\, s_x\, s_y\, r}$$

$$(6)\quad g = (r/|r|)(s_y/s_x).$$

These regression lines all pass through the centroid $\bar{x},\bar{y}$ of the x,y distribution, so the corresponding intercepts $a = \ln k$ in Huxley's law are given by

$$(7)\quad a = \bar{y} - [b,\ m,\ \text{or}\ g]\bar{x}.$$

Except when $|r|$ is very close to 1, the slopes, and hence also the physical implications, of the regression lines are different, as shown by Seim and Saether (1983). To further elucidate this important point, we consider the relative magnitudes of the regression coefficients as a function of the measurement parameters $|r|$ and $u = s_y/s_x$ for the ranges $0 < |r| < 1$ and $0 < u < \infty$. We note first that b, m, and g all have the same algebraic sign as r and that $|b| < |g|$, which follows from inspection of 4–6. From Fig. 1a, for any fixed u, m/b exceeds 1 by an amount that increases as $|r|$ decreases. For u greater than 1/2 and $|r|$ of the order of 1/2 or less, say, m/b may be substantially larger than 1. Thus, e.g., b-values less than 1 indicating negative allometric growth, and m-values greater than 1 indicating positive allometric growth, can occur for certain data sets, as shown below. The plot of m/g in Fig. 1b shows that for a given $|r|$, m/g may be less than, equal to, or greater than 1, depending on u. Except when $u = 1$, for which $m = g = r/|r| = \pm 1$, the differences between m and g are seen to grow as $|r|$ decreases.

The relative magnitudes of the estimators for $0 < |r| < 1$ and $0 < u < \infty$ are summarized as follows:

$$(8)\quad |m| > |g| > |b| \text{ for } u > 1$$

$$(9)\quad |m| = |g| = 1 > |b| = |r| \text{ for } u = 1$$

$$(10)\quad |g| > |m| > |b| \text{ for } u < 1.$$

Also,

$$(11)\quad b,\ m,\ g \to \pm\, s_y/s_x \text{ as } r \to \pm\, 1.$$

Since $|r|$ often differs appreciably from 1 in allometric measurements, substantial systematic differences between b, m, and g can occur. To facilitate the recognition of trends in q for different species, only results obtained with the same estimator should be compared.

The linear structural model and analysis limitations — When the presence of biological variability and measurement error in both x and y is taken into account, it is found that the constants q and a in Huxley's law can only be approximated, regardless

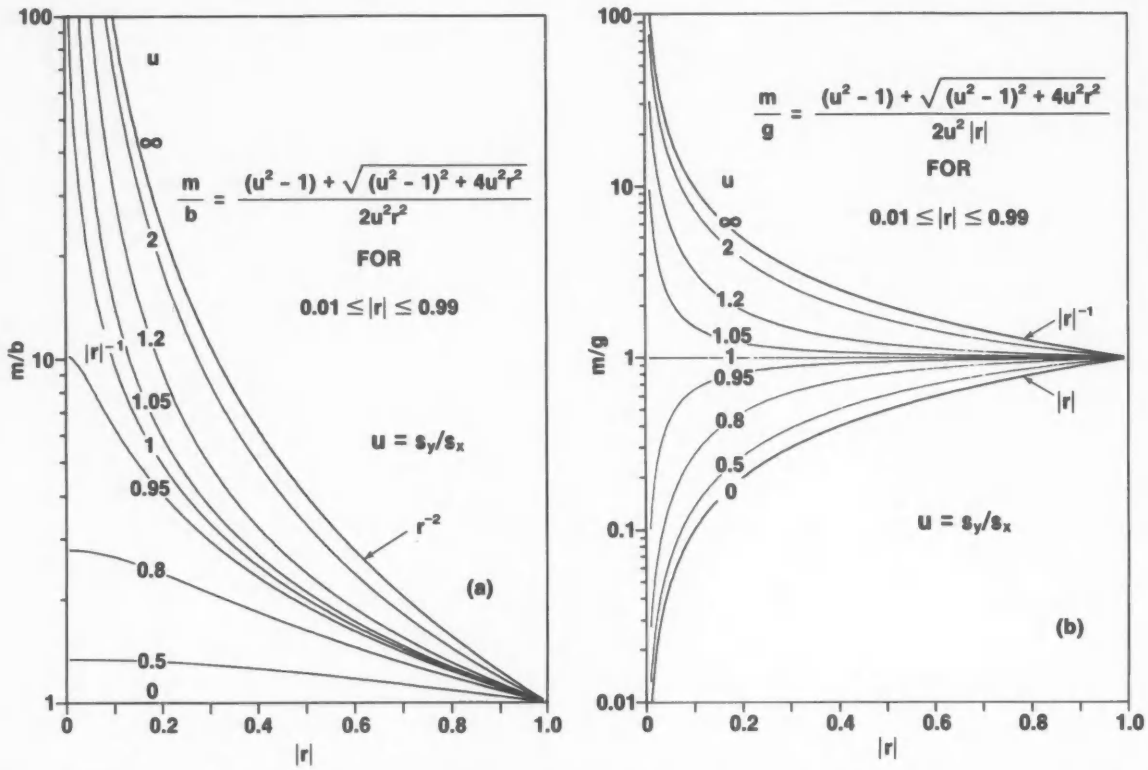

FIG. 1. Theoretical relationships between the respective y-on-x regression, major axis, and reduced major axis estimators b, m, and g for the allometric exponent. Plots of m/b in (a) and m/g in (b) are given as functions of sample values $|r|$ and $u = s_y/s_x$. As $|r| \to 1$, b, m, and $g \to \pm s_y/s_x$, according to the sign of r. For $|r|$ not close to 1, the estimators are usually dissimilar and have different physical implications.

of the number of data points available. In the linear structural model (Kendall and Stuart 1979, ch. 29; Kuhry and Marcus 1977) measurements x,y are represented in the form

$$(12) \qquad \begin{aligned} x &= x' + \delta \\ y &= y' + \epsilon \end{aligned}$$

in which x' and y' are unobservable random variables which exactly obey Huxley's law

$$(13) \qquad y' = a + q\,x'$$

and δ,ϵ are fluctuation components due to individual biological variability and random measurement error. In this model, Huxley's law is more precisely defined as a "linear structural relation" between the structural variables x' and y', rather than the observed variables x and y. The residual fluctuations δ and ϵ are assumed to be independent random variables with zero means and respective variances σ^2_δ and σ^2_ϵ. These fluctuations are assumed to be uncorrelated with each other and with either of the random variables x', y'. With the additional assumption that x', y', δ and ϵ are normally distributed, x,y are bivariate normal. We tacitly assume that δ and ϵ are due mainly to biological variability, although some measurement error is undoubtedly present.

With these assumptions the maximum likelihood estimator for q is found to be

$$(14) \qquad \hat{q}(\lambda) = \frac{(s_y^2 - \lambda\, s_x^2) + \sqrt{(s_y^2 - \lambda\, s_x^2)^2 + 4\,\lambda\, s_x^2\, s_y^2\, r^2}}{2\, s_x\, s_y\, r}.$$

where $\lambda = \sigma^2_\epsilon/\sigma^2_\delta$. The estimators b, m, and g are given by (14) when λ has the respective values ∞, 1, and s_y^2/s_x^2, i.e., $b = \hat{q}(\infty)$, $m = \hat{q}\,(1)$, and $g = \hat{q}(s_y^2/s_x^2)$. Also, for $\lambda = 0$, $\hat{q}(0) = s_y/(s_x r) = b'$, the reciprocal of the x-on-y regression coefficient (Kendall and Stuart 1979; Jolicoeur and Heusner 1971; Kuhry and Marcus 1977). It can be shown that

$$(15) \qquad |b| \leq |\hat{q}(\lambda)| \leq |b'|$$

so long as $s_x s_y r \neq 0$ (Kendall and Stuart 1979).

Since λ is unknown, the ML estimator $\hat{q}(\lambda)$ cannot be specified. The ambiguity involved in selecting an estimator for q is discussed by Jolicoeur and Heusner (1971). Using the linear structural model, logarithmic variables, and various assumed values for λ in 14, they derived hypothetical ML estimates of the weight exponent for standard metabolism in white rats and also the 99% confidence intervals associated with the estimates. For $2/3 \leq \lambda \leq 3/2$, the confidence intervals (which included both point estimates $m = 0.70$ and $g = 0.74$) all contained the range of exponent values 2/3 – 3/4 expected for mammals from numerous studies (Schmidt-Nielsen 1984). This exponent range was not contained within the confidence intervals for the point estimates b and b' which are biased, since they do not include

the effects of variability in both animal weight x and O_2-consumption rate y. For this particular data set, Jolicoeur and Heusner concluded that the correct value for λ is probably near 1, somewhere between 2/3 and 3/2.

For an arbitrary bivariate normal data set it is seen that, without additional physical information, one can at most postulate an interval containing the λ value defining $\hat{q}(\lambda)$. The inability to provide a consistent estimator for q is an inherent limitation of bivariate allometry. To overcome this limitation, certain additional measurements are required that can rarely be performed (Kendall and Stuart 1979; Kuhry and Marcus 1977).

In practice, when variability is believed to be present in both x and y, the slope of either the major axis or the reduced major axis is used to estimate q. The relative merits of the estimators m and g have been warmly debated (Ricker 1973, 1975; Jolicoeur 1975). However, from the perspective of the linear random model in which q is estimated by $\hat{q}(\lambda)$ in 14, and from the preceding discussion, it appears that the choice is somewhat arbitrary. We favor the major axis estimator m in this paper partly because of the simplicity of the assumption $\lambda = 1$ defining m as compared with the assumption $\lambda = s_y^2/s_x^2$ defining g and partly because results obtained with it appear to be particularly significant physically.

Dimensionless allometric law structural relations — We have seen that Huxley's allometric law is to be interpreted as a linear structural relation

$$(16) \quad y' = a + q\,x'$$

between the logarithmic structural variables x' and y'. The intercept a is given by

$$(17) \quad a = \overline{y'} - q\,\overline{x'},$$

since the centroid $\overline{x'}$, $\overline{y'}$ must lie on the line 16. In terms of the linear structural variables l' and R' defined by $x' = \ln(l'/l_0)$ and $y' = \ln(R'/l_0)$, the law is

$$(18) \quad R'/l_0 = k(l'/l_0)^q$$

with

$$(19) \quad k = e^a.$$

The allometric exponent q is the ratio of the relative growth rates of R' and l' (Huxley 1932, 1972):

$$(20) \quad q = \frac{1}{R'}\left(\frac{dR'}{dt}\right) \Big/ \frac{1}{l'}\left(\frac{dl'}{dt}\right).$$

While the dimensionless constant q is independent of the length unit l_0, k and $a = \ln k$ are not, so they are not usually biologically significant (Lumer et al. 1942; White and Gould 1965). To obtain physically meaningful constants k and a for interspecific comparison of growth trends, the reference variable l must be expressed in terms of a length unit characterizing a common phase of swimbladder development (Lumer et al. 1942).

Physical considerations suggest that as a swimbladdered fish approaches its maximum size, the percentage swimbladder volume 100 V_B/V_F, where V_F is fish total volume, may approach some characteristic limit for each species. Here, where the reference variable is fish standard length rather than volume, we have scaled l-values for each species to the maximum length l_{max} in its data set. So that all terms in the resulting allometric law would be dimensionless, R-values were also scaled to l_{max}.

Introducing the dimensionless linear structural variables $l'_* = l'/l_{max}$ and $R'_* = R'/l_{max}$ and their logarithmic forms $x'_* = \ln l'_*$ and $y'_* = \ln R'_*$, equations 16 and 17 transform to

$$(21) \quad y'_* = a_* + q\,x'_*$$

and

$$(22) \quad a_* = \overline{y'_*} - q\,\overline{x'_*}$$

while 18 and 19 transform to

$$(23) \quad R'_* = k_*(l'_*)^q$$

with

$$(24) \quad k_* = e^{a_*}.$$

The relation between k_* and k is

$$(25) \quad k_* = k(l_{max}/l_0)^{q-1}$$

from which $a_* = \ln k_*$ may be expressed in terms of $a = \ln k$. We term the structural relations 16 and 18 dimensional forms of Huxley's allometric law and the relations 21 and 23 dimensionless forms of the law. In the a, a_* intercept formulae 17 and 22, sample means of the structural variables may be replaced by sample means of the corresponding measured variables, since the expected means of δ and ϵ in 12 are zero. It is seen that the linear structural relations 16 and 21 are estimated by regression lines derived, respectively, from dimensional x,y or dimensionless x_*,y_* data.

Theoretical structural relations for nonregressive species — Since all random variables in the following discussion are structural variables, primes are omitted for notational convenience. As a nonregressive swimbladdered fish grows, its average tissue density increases with the development of the skeleton, gonads, and muscle tissue. The allometric growth of the swimbladder may be supposed to be a mechanism for providing progressively larger contributions to positive buoyancy to compensate, at least in part, for this increase in tissue density. Specialized gas secretion and resorption tissues and supportive capillaries, all intimately associated with the swimbladder wall, are involved in this task (Fänge 1953; Marshall 1960; Alexander 1966).

Consider now the theoretical structural relation between R and l resulting from the hypothesis that, for the swimbladder and associated tissues to provide the necessary buoyancy contributions during fish growth, swimbladder wall area S_B must increase in proportion to M^θ, where M is fish mass and θ is a species-specific constant close to an ideal theoretical value of 1. In order to estimate θ from available data, however, it is necessary to assume instead that $S_B \sim V_F^{\,\theta}$, which is nearly equivalent, since fish volume $V_F = M[\rho_t(1 - V_B/V_F)]^{-1} \sim M$ to a first approximation in which average tissue density ρ_t is considered constant during fish growth and V_B/V_F is neglected compared with 1. For mathematical simplicity, we assume an "ideal" nonregressive swimbladdered species with a thin-walled, gas-filled swimbladder in the shape of a prolate spheriod. In addition, we assume that swimbladder shape is geometrically similar for mature specimens of each species so that the swimbladder axes ratio $v = b/a$ is a species-specific constant independent of fish length l. Plots of b/a vs l for a representative myctophid (**Lobianchia dofleini*, gonostomatid (**Vinciguerria attenuata*), sternoptychid (*Sternoptyx diaphana*) and melamphaid (*Melamphaes typhlops*) suggest this is a reasonable physical approximation.

TABLE 2. Maximum likelihood estimates of the parameters $[\bar{x}, \bar{y}, s_x, s_y, r]$ defining sample bivariate normal distributions $p(x, y)$ for the 38 swimbladdered fish species listed in Table 1. The quantities b, m, and g are the slopes, respectively, of the y-on-x regression line, major axis (orthogonal regression line), and reduced major axis calculated from the ML values.

Species no.	Name	n	$\bar{x}$	$\bar{y}$	s_x	s_y	r	b	m	g
					MYCTOPHIDAE					
1	BSUB	11	2.976	−0.3697	0.2907	0.5856	0.8225	1.657	2.284	2.014
2	BIND	31	3.301	−0.0971	0.1771	0.3186	0.6350	1.142	2.378	1.799
5	CWAR	37	3.619	−0.1566	0.3849	0.3927	0.6171	0.630	1.033	1.020
7	DMET	11	4.059	1.0123	0.3870	0.5083	0.9033	1.186	1.351	1.313
10	*DRAF	48	3.868	0.7707	0.3880	0.5047	0.9401	1.223	1.322	1.301
11	DATL	32	2.650	−0.8018	0.1458	0.2926	0.7944	1.594	2.329	2.007
12	*HBEN	65	2.936	−0.4304	0.5345	0.5969	0.9076	1.014	1.129	1.117
13	*HHYG	51	3.635	−0.0035	0.3287	0.4097	0.5181	0.646	1.517	1.246
14	HTAA	15	3.048	−0.6334	0.3644	0.4813	0.7820	1.033	1.424	1.321
18	LURO	35	3.851	0.1079	0.3557	0.5130	0.7710	1.112	1.597	1.442
19	LALA	11	3.660	−0.0076	0.1508	0.2995	0.6173	1.226	2.765	1.987
20	†LATE	35	3.796	−0.9143	0.3416	0.3180	−0.4641	−0.432	−0.858	−0.931
21	*LCRO	63	3.827	0.1337	0.6773	0.8161	0.9700	1.169	1.212	1.205
22	†LCUP	44	3.926	−1.3352	0.2443	0.1897	−0.6189	−0.480	−0.669	−0.776
23	LFES	37	3.678	−0.0864	0.2814	0.3874	0.8178	1.126	1.474	1.377
25	LPHO	43	3.668	0.0133	0.2772	0.3547	0.6966	0.892	1.420	1.280
26	*LPUS	47	3.280	−0.3046	0.3082	0.3908	0.8901	1.129	1.305	1.268
27	LGAU	32	3.293	−0.3474	0.3522	0.5238	0.8266	1.229	1.608	1.487
29	*LDOF	57	3.218	−0.3687	0.2981	0.3863	0.6367	0.825	1.493	1.296
30	LGEM	42	3.275	−0.1679	0.4436	0.4947	0.8887	0.991	1.130	1.115
34	NRES	60	3.594	−0.3090	0.4320	0.4385	0.6343	0.644	1.024	1.015
35	SRUF	25	3.591	−0.3000	0.3899	0.6194	0.7302	1.160	1.853	1.589
36	TBAT	25	3.819	0.4051	0.3950	0.5445	0.9456	1.304	1.403	1.379
37	TMIN	30	3.611	0.0502	0.2631	0.4414	0.8493	1.425	1.822	1.678
					GONOSTOMATIDAE					
38	BPED	26	3.729	0.3504	0.2541	0.3919	0.8631	1.332	1.644	1.543
39	*IOVA	25	3.184	0.0568	0.3535	0.5431	0.9658	1.484	1.559	1.536
41	VTRI	71	3.072	−0.1057	0.1739	0.2927	0.8564	1.442	1.821	1.684
42	*VATT	41	2.980	−0.6175	0.3282	0.4342	0.9081	1.201	1.360	1.323
43	*VPOW	29	3.034	−0.4947	0.2790	0.3574	0.8242	1.056	1.348	1.281
					STERNOPTYCHIDAE					
44	AACU	30	2.752	−0.3582	0.3818	0.4758	0.9373	1.168	1.265	1.246
45	*AHEM	47	2.929	−0.1420	0.3963	0.4827	0.9658	1.177	1.227	1.218
46	SDIA	30	2.967	0.0303	0.3428	0.4560	0.9598	1.277	1.346	1.330
					MELAMPHAIDAE					
47	MPUM	59	2.863	−0.4821	0.1745	0.3007	0.9017	1.554	1.818	1.723
48	MTYP	30	3.480	0.1965	0.5134	0.6381	0.9455	1.175	1.258	1.243
49	SOPI	30	3.294	−0.1099	0.2795	0.3406	0.8842	1.077	1.250	1.219
51	PCAP	32	3.376	−0.1235	0.6108	0.7395	0.9396	1.138	1.225	1.211
					ANOPLOGASTERIDAE					
50	ACOR	5	3.385	−0.1231	0.7107	0.3766	0.9911	0.525	0.527	0.530
					OPISTHOPROCTIDAE					
55	RNAT	9	3.459	−0.0585	0.2976	0.3920	0.9013	1.188	1.357	1.318

From the formula for the area of a prolate spheroid, the outside wall area S_B of the swimbladder may be expressed as

$$S_B = 4\pi R^2 Z(v) \quad (26)$$

where

$$Z(v) = \frac{v^{2/3}}{2}\left[1 + \frac{\arcsin(\sqrt{1 - v^2})}{v\sqrt{1 - v^2}}\right]. \quad (27)$$

Z has the value 1 for $v = 1$ and gradually increases as v decreases: $Z = 1.37$ for $v = 1/5$ and $Z = 1.70$ for $v = 1/10$, e.g. Thus, $S_B \sim R^2$ as for a spherical swimbladder, but with a constant of proportionality $4\pi Z$ depending on the species. Our hypothesis $S_B \sim V_F^{\theta}$ then takes the form $R^2 \sim V_F^{\theta}$ which implies the structural relation

$$R/l_0 = \text{const}\,(V_F/V_0)^{\theta/2} \quad (28)$$

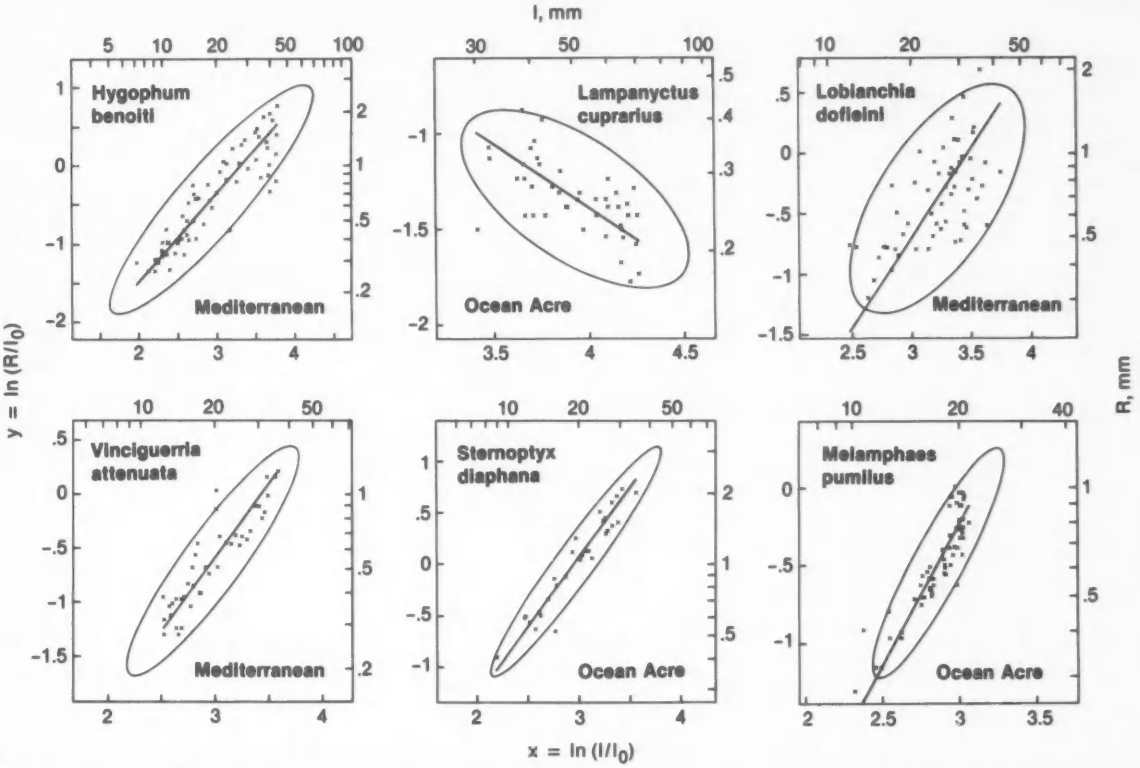

FIG. 2. Swimbladder size allometry data in the linear allometric law range for six species found at Ocean Acre: **Hygophum benoiti* (12), †*Lampanyctus cuprarius* (22), **Lobianchia dofleini* (29), **Vinciguerria attenuata* (42), *Sternoptyx diaphana* (46), and *Melamphaes pumilus* (47). Unit lengths on the x, y axes are equal, but different for each species. The mean $\bar{x}$, $\bar{y}$ is located at the center of each plot. Major axes and 95% probability ellipses were constructed from the distribution parameters in Table 2. As seen from the slopes of the major axes, except for †*L. cuprarius* (22), the species have nonregressive swimbladder growth characteristics. The scattergrams for **L. dofleini* (29), **V. attenuata* (42), and *S. diaphana* (46) pass all tests for bivariate normality described in the text.

with V_0 unit dimensional volume.

Corresponding to the analogous mass-length relation for fish (LeCren 1951), we take

$$(29)\quad V_F/V_0 = k_1(l/l_0)^{q_1},$$

where k_1 and q_1 are constants. Finally, substituting V_F/V_0 from (29) into (28), we obtain the theoretical structural relation

$$(30a)\quad R/l_0 = \text{const}\ (l/l_0)^{\theta(q_1/2)},\ \theta \approx 1.$$

Since R/l_0 is proportional to $(l/l_0)^q$ by Huxley's law, we have also

$$(30b)\quad q = \theta(q_1/2).$$

Qualitatively, one expects $q_1 \approx 3$ for most species. If θ is distributed reasonably closely about the ideal theoretical value 1, then $q \approx 3/2$ from 30b. As may be inferred from 30a, dimensionless allometric law lines 21 for nonregressive species should be approximately parallel with an average slope of about 3/2.

Results and Discussion

Bivariate Normal Models and Tests for Bivariate Normality

Bivariate normal $p(x,y)$ models have been developed for the 38 swimbladdered species in Table 1. ML estimates of the distribution parameters for each species are given in Table 2. Species names in this table are represented by a 4-letter acronym consisting of the first letter of the genus and the first three letters of the species name. In the following discussion, species numbers in parentheses follow species names to facilitate the location of tabulated information.

Scattergram plots for 6 of the 38 species are shown in Fig. 2. The straight line through each data set is the orthogonal regression line (major axis of $p(x,y)$) over the x-range of the data. The 95% equiprobability ellipse for the sample distribution is also drawn.

Selected x,y populations were tested for bivariate normality by implementing the tests described above in the form of χ^2-tests. The sample values x and y were tested for normality assuming they can be described by $N(\bar{x},s_x^2)$ and $N(\bar{y},s_y^2)$, respectively. The distance d of an x,y point from the orthogonal regression line was assumed to be a normal variate described by $N(0, \overline{d^2})$, where $\overline{d^2}$ is the mean square of d. $Q(x,y)$ was assumed to have a χ^2-distribution with 2 degrees of freedom, neglecting errors resulting from the use of ML estimates rather than the unknown population parameters in Eq. (2). In computing χ^2-values for each test, the variates x, y, d, and Q were partitioned into 5 class intervals of equal theoretical

TABLE 3. Results of χ^2-tests for 30 nonregressive and 2 regressive swimbladdered fish species with $n \geq 25$. The hypotheses tested are that the variables x, y, and the deviation distance d of a point from the orthogonal regression line are each normal variates and that $Q(x, y)$ is a χ^2-variate with 2 degrees of freedom. These hypotheses are true if the x, y distribution is bivariate normal. P indicates acceptance of an individual hypothesis at the 5% significance level while F(AS), F(S), and F(HS) denote increasingly significant deviations from the hypothesis (see text).

Species no.	Name	n	x	y	d	$Q(x, y)$
			MYCTOPHIDAE			
2	BIND	31	P	P	P	P
5	CWAR	37	P	P	P	P
10	*DRAF	48	F(HS)	P	P	P
11	DATL	32	P	P	P	P
12	*HBEN	65	F(AS)	P	P	P
13	*HHYG	51	F(HS)	P	P	P
18	LURO	35	F(AS)	P	P	P
20	†LATE	35	P	P	P	P
21	*LCRO	63	P	P	P	P
22	†LCUP	44	F(AS)	P	P	P
23	LFES	37	P	P	P	P
25	LPHO	43	P	P	P	P
26	*LPUS	47	P	F(AS)	P	P
27	LGAU	32	F(S)	P	P	P
29	*LDOF	57	P	P	P	P
30	LGEM	42	P	P	P	P
34	NRES	60	F(HS)	P	P	P
35	SRUF	25	P	P	P	P
36	TBAT	25	F(HS)	F(HS)	P	P
37	TMIN	30	P	P	P	P
			GONOSTOMATIDAE			
38	BPED	26	P	F(S)	P	P
39	*IOVA	25	F(HS)	F(HS)	P	P
41	VTRI	71	F(HS)	F(HS)	P	F(AS)
42	*VATT	41	P	P	P	P
43	*VPOW	29	P	F(AS)	P	P
			STERNOPTYCHIDAE			
44	AACU	30	P	P	P	P
45	*AHEM	47	P	P	P	P
46	SDIA	30	P	P	P	P
			MELAMPHAIDAE			
47	MPUM	59	P	P	P	F(S)
48	MTYP	30	P	P	P	P
49	SOPI	30	F(AS)	P	P	P
51	PCAP	32	P	F(HS)	P	P

probability based on these hypothetical distributions. Since the expected number $n/5$ of occurrences in each class interval must be at least 5 for the χ^2-test to be valid, only species with $n \geq 25$ were tested for bivariate normality in this manner. The χ^2-test statistic has 2 degrees of freedom for the x and y tests, 3 for the d test, and 4 for the Q test. All tests were at the 5% significance level.

Following the convention of Cramér (1946), a test result is considered consistent with the null hypothesis if $\chi^2 \leq \chi^2_{0.05}$. When $\chi^2_{0.05} < \chi^2 \leq \chi^2_{0.01}$, there is an "almost significant (AS)" deviation from the hypothesis. If $\chi^2_{0.01} < \chi^2 \leq \chi^2_{0.001}$, the deviation is "significant (S)," and if $\chi^2_{0.001} < \chi^2$, the deviation is "highly significant (HS)." We let P denote passage of the test for acceptance of the null hypothesis and F(AS), F(S), and F(HS) denote failures of the test at the respective

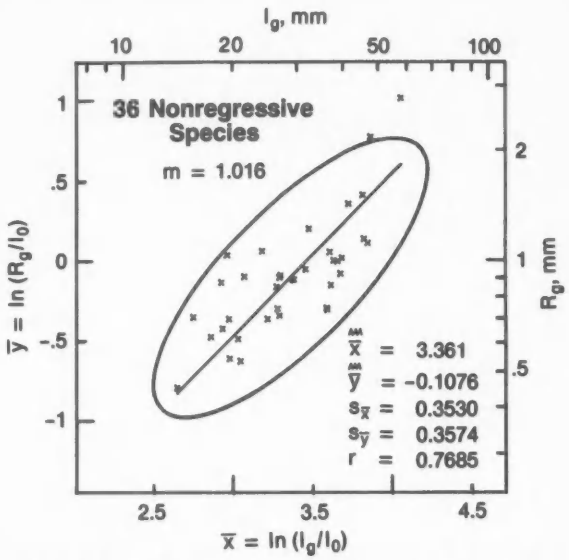

FIG. 3. Relationship between the mean logarithmic size variables $\bar{x} = \ln(l_g/l_0)$ and $\bar{y} = \ln(R_g/l_0)$ for the 36 nonregressive swimbladdered species group. ML estimates of the distribution parameters and the major axis slope m are given in the figure. The distribution is found to be bivariate normal. A portion of the major axis and the 95% equiprobability ellipse of the distribution are drawn.

levels of significance just defined. The results of these tests for the 32 swimbladdered species in Table 1 with $n \geq 25$ are given in Table 3.

For 22 of the 32 species, it is seen that at the 5% level, there are no significant failures in any of the four tests. Scattergrams of 15 nonregressive and 1 regressive species, exactly half of those tested, passed all four tests for bivariate normality. The fact that x-test failures are relatively more common and serious is perhaps to be expected, since the length variable x is most directly affected by sampling variability and bias in biological trawls and also human bias in the selection of samples for dissection. For 7 of the 10 species with one or more significant failures, the appearance of the x,y scattergrams suggest that sampling fluctuations in x are probably the underlying cause. For the 16 species whose scattergrams failed one or more of these tests and for the 6 species with insufficient data to be tested, we may regard the parameters in Table 2 as defining "effective" bivariate normal models.

Test failures not obviously due to sampling deficiencies can sometimes be traced to biological factors. Thus, referring to Table 2 and Fig. 2, the x test failure for †*Lampanyctus cuprarius* (22) is probably due to sampling variability, whereas the x test failure for **Hygophum benoiti* (12) and the Q test failure for *Melamphaes pumilus* (47) are undoubtedly due, at least in part, to "specific anomalies." In the *H. benoiti* (12) scattergram, the increased downward scatter in y for large specimens indicates the presence of some individuals with swimbladders of a different morphological type, a condition found to occur in certain myctophid species. Kleckner and Gibbs (1972) and Kleckner (1974) found that contracted as well as the usual inflated swimbladders occurred in the larger size specimens of the following Mediterranean myctophid species in Table 3: **Hygophum benoiti* (12), **H. hygomi* (13), **Lampanyctus*

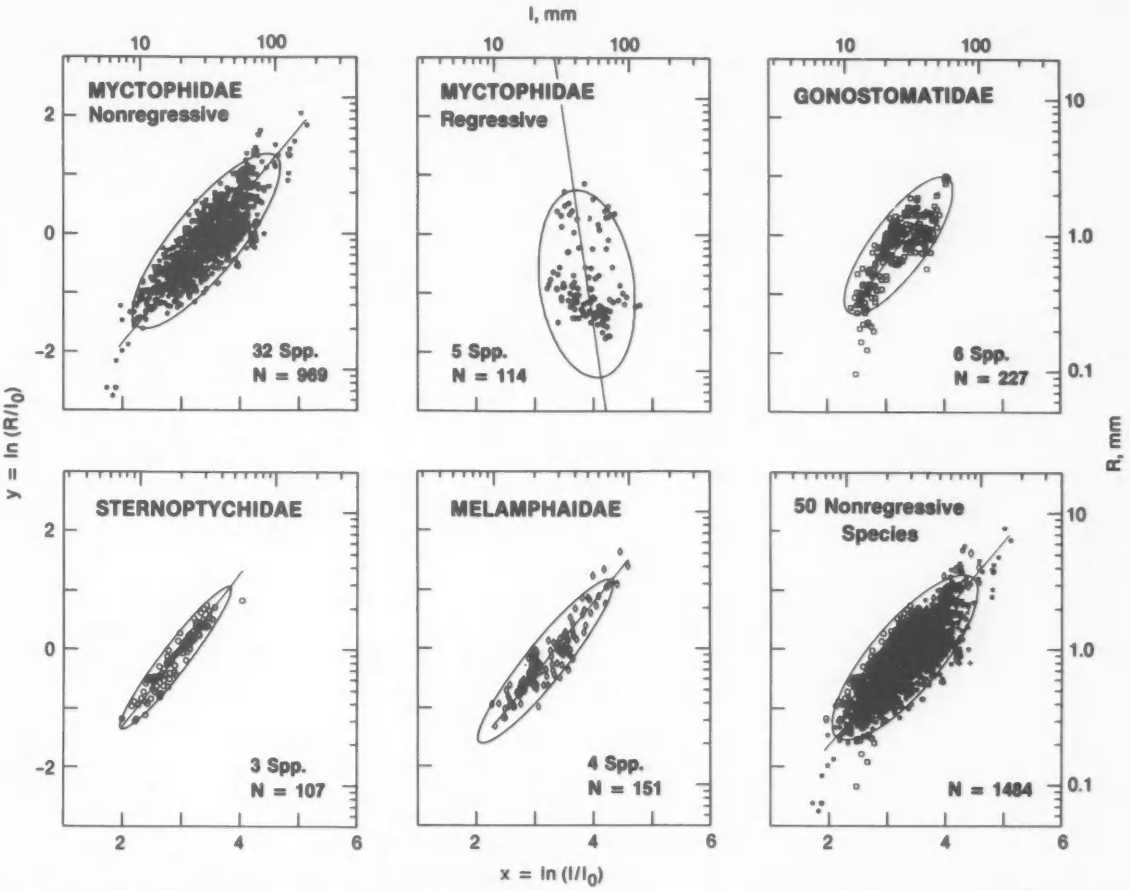

FIG. 4. Swimbladder size distributions for six swimbladdered fish species groups that contribute to acoustic backscattering in the North Sargasso Sea. Data for 55 swimbladdered species are represented, including x, y points for immature specimens that do not follow the allometric law. A portion of the major axis and the 95% equiprobability ellipse are drawn for the distribution of mature specimens in each group using the parameters in Table 4.

crocodilus (21), **L. pusillus* (26), and **Lobianchia dofleini* (29). Due to the contracted swimbladder data component and sampling fluctuations in x, the **H. benoiti* (12) distribution fails the x-test "almost significantly." In the **H. hygomi* (13) scattergram (not shown), sampling happens to be such that this component is strongly emphasized, and the failure of the x-test is "highly significant." Contracted swimbladder data in the scattergrams of **Lobianchia dofleini* (29) in Fig. 2 and **Lampanyctus crocodilus* (21) and **L. pusillus* (26) (neither shown) are difficult to distinguish from inflated swimbladder data, and indeed all three distributions pass the x-test. In fact, the **Lobianchia dofleini* (29) and **Lampanyctus crocodilus* (21) distributions pass all four statistical tests. For species which exhibit both inflated and contracted swimbladders, a different allometric model is required. However, when the contracted swimbladder component is relatively small, we see from these examples that a simple bivariate normal model may still provide an adequate overall description of x,y distribution properties.

The *Melamphaes pumilus* (47) distribution in Fig. 2 fails the Q-test significantly, presumably due to the sudden upward trend of the data at the large-size end of the distribution. Much less marked upward trends are also discernible in the scattergrams of two other of our four melamphaid species, *Melamphaes typhlops* (48) and *Scopeloberyx opisthopterus* (49). The fourth melamphaid, *Poromitro capito* (51), has a single highly significant failure in the y test not simply due to sampling fluctuations, as is revealed by an inspection of its scattergram. At the small-size end of the x,y distribution, there is a short, well-defined flat portion between 13 and 17 mm which changes abruptly to a linear trend with positive slope as x increases. Although data for the smaller sizes are sparse, a similar feature appears faintly in the *M. typhlops* (48) distribution. These, and possibly other, melamphaid species have nonlinear growth trends. From the fact that *M. typhlops* (48) passes all the statistical tests, we see that a bivariate normal model may suffice for species in which nonlinear departures from Huxley's allometric law are relatively small.

It is of some interest to note that the means $\bar{x}$, $\bar{y}$ for the 36 nonregressive species are also described by a bivariate normal distribution. The $\bar{x}$, $\bar{y}$ scattergram for these species, which

TABLE 4. Effective bivariate normal models for various swimbladdered fish species groups. Only data for mature specimens were used in estimating sample parameters. Except for the regressive myctophid group, all groups consist of nonregressive swimbladdered species. Slopes of the y-on-x regression line, major axis, and reduced major axis are also given together with the standard length limits for each distribution.

	Myctophidae Nonreg., 32 spp.	Myctophidae Reg., 5 spp.	Gonostomatidae 6 spp.	Sternoptychidae 3 spp.	Melamphaidae 4 spp.	Nonregressive 50 spp.
N	969.	114.	227.	107.	151.	1484.
$\bar{x}$	3.454	3.914	3.232	2.890	3.180	3.351
$\bar{y}$	−0.1347	−0.8399	−0.1654	−0.1543	−0.1973	−0.1483
s_x	0.5150	0.3371	0.3816	0.3879	0.4761	0.5136
s_y	0.6048	0.6481	0.4735	0.4954	0.5660	0.5749
r	0.8223	−0.2114	0.7877	0.9525	0.9295	0.7968
b	0.966	−0.406	0.978	1.217	1.105	0.892
m	1.215	−6.782	1.314	1.293	1.204	1.152
g	1.174	−1.923	1.241	1.277	1.189	1.119
l_{min}(mm)	7.2	25.2	11.2	7.2	9.6	7.2
l_{max}(mm)	171.7	121.9	59.0	55.9	99.1	171.7

passed all four statistical tests, is seen in Fig. 3. The nearly isometric relation between the geometric means R_g and l_g implied by the closeness of m to 1, however, is not typical of the allometric growth of R with l for most individual species, as shown below.

Mathematical models for the x,y distributions of species groups are of potential usefulness in bioacoustic modeling. At Ocean Acre, 55 swimbladdered fish species including the 38 species treated here are the principal sound scatterers at frequencies below 20 kHz (Brown and Brooks 1974). Using all available x,y data for the 55 species, scattergrams were constructed for the leading families of swimbladdered fish at Ocean Acre, and also one for 50 nonregressive species believed to be responsible for most of the observed scattering. These are shown in Fig. 4. Distributions obtained by pooling data in this manner will not usually be bivariate normal. The distributions in Fig. 4 are not, with the exception of that for the Sternoptychidae which, rather curiously, passed all four tests for bivariate normality with 10 equal probability bins for the χ^2-tests. The constants in Table 4 are a first attempt to define effective bivariate normal models for these species groups. For a full description of the application of bivariate normal models to biacoustic modeling, see Saenger (1988).

Allometric Exponents and Structural Relations

Allometric exponents from major axis, reduced major axis, and regression analyses; confidence limits — Allometric exponents b, m, and g for the 38 swimbladdered species are given in Table 2. Differences in exponent values for a given species, which may be considerable depending on $|r|$ and $u = s_y/s_x$, are simply explained with the help of Fig. 1 and the inequalities 8–11.

Consider first the 36 nonregressive species with positive exponents. For the 35 nonregressive species with $u = s_y/s_x > 1$ (all except *Anoplogaster cornuta* (50)), $m > g > b$, as expected from the inequality 8 above. For two of the species, *Ceratoscopelus warmingi* (5) and *Notoscopelus resplendens* (34), u is near 1, and as expected from (9), m and g values are approximately 1, while b is about 0.63–0.64, close to the r values for the two species. Thus, the regression coefficient b indicates negative allometric growth while m and g both indicate nearly isometric growth. More striking differences, where m and g indicate positive allometric growth, while b indicates negative allometric growth, are found notably for **Hygophum hygomi* (13), *Lampanyctus photonotus* (25) and **Lobianchia dofleini* (29). The single nonregressive species *A. cornuta* (50) with a u value less than 1, viz. $u = 0.53$, has a correlation coefficient r very near 1, $r = 0.99$. As expected from (10) and (11), $g > m > b$, with each value approximately equal to 0.53. Values of b, m, and g are also close for other nonregressive species with r very near 1, e.g., **Lampanyctus crocodilus* (21), **Ichthyococcus ovatus* (39), **Argyropelecus hemigymnus* (45), and *Sternoptyx diaphana* (46), for which $0.96 \leq r \leq 0.97$. The two regressive species, †*Lampanyctus ater* (20) and †*L. cuprarius* (22) have $u < 1$, and $|g| > |m| > |b|$, as expected from (10). For †*L. ater* (20), $|r| = 0.46$ is relatively small, and bladder regression implied by m and g is much more severe than that implied by b. Despite the low value of $|r|$, m and g are fairly close since $u = 0.93$ is not far from 1.

Table 5 contains 95% confidence limits for m, b, and g, lower and upper limits being indicated, respectively, by subscripts 1 and 2. Using the sample parameters in Table 2, confidence limits for b were computed from standard formulae given, e.g., by Cramér (1946). Approximate small sample confidence limits for m were computed from formulae given by Jolicoeur (1973) and those for g from formulae given by Jolicoeur and Mosimann (1968). Point estimates m and g for all nonregressive species except *Anoplogaster cornuta* (50) exceed 1. Also, the corresponding 95% confidence intervals for both m and g lie above 1 for all but seven of these species. This suggests that allometric growth is positive in most, if not all, of the 35 nonregressive species. Allometric growth in *A. cornuta* (50) is negative, indicated by the fact that both the point values of m and g and their confidence intervals lie below 1.

Swimbladder growth trends characterized with the dimensionless allometric law — Allometric law constants k, m, and k_* obtained from major axis analysis are given in Table 6 for the 38 swimbladdered species. The unit-dependent constant k in the dimensional form of the law has a wide range of variation for the 36 nonregressive species, from $4.003(10^{-5})$ for *Lampanyctus alatus* (19) to $1.484(10^{-1})$ for *Anoplogaster cornuta* (50). In contrast, the constant k_* in the dimensionless form of the law has a small range of variation, $1.853(10^{-2})$ for *A. cornuta* (50) to $6.503(10^{-2})$ for *Sternoptyx diaphana* (46). Slopes m range from 0.527 for *A. cornuta* (50) to 2.765 for *L. alatus* (19). Corresponding constants for the two regressive species †*Lampanyctus ater* (20) and †*L. cuprarius* (22) are qualitatively very different, as might be expected.

Plots of the dimensionless allometric law structural relation

TABLE 5. Ninety-five percent confidence limits for the slopes of the y-on-x regression line, major axis, and reduced major axis, respectively, for 38 Ocean Acre swimbladdered fish species. Point estimates b, m, and g for the slopes are given in Table 2.

		y-on-x regression		Major axis		Reduced major axis	
Species no.	Name	b_1	b_2	m_1	m_2	g_1	g_2
			MYCTOPHIDAE				
1	BSUB	0.793	2.521	1.433	4.555	1.328	3.056
2	BIND	0.615	1.670	1.573	4.274	1.347	2.402
5	CWAR	0.354	0.905	0.648	1.657	0.781	1.332
7	DMET	0.762	1.611	0.948	2.006	0.956	1.805
10	*DRAF	1.091	1.354	1.189	1.475	1.176	1.439
11	DATL	1.140	2.049	1.789	3.216	1.603	2.513
12	*HBEN	0.896	1.132	1.005	1.271	1.005	1.241
13	*HHYG	0.340	0.952	0.955	2.677	0.977	1.590
14	HTAA	0.540	1.526	0.887	2.508	0.917	1.903
18	LURO	0.787	1.437	1.205	2.202	1.153	1.804
19	LALA	0.048	2.405	1.241	62.384	1.131	3.489
20	†LATE	−0.724	−0.140	−1.847	−0.357	−1.268	−0.684
21	*LCRO	1.094	1.244	1.137	1.293	1.132	1.282
22	†LCUP	−0.670	−0.291	−0.982	−0.426	−0.989	−0.609
23	LFES	0.854	1.398	1.165	1.907	1.132	1.675
25	LPHO	0.602	1.181	1.033	2.027	1.023	1.602
26	*LPUS	0.955	1.302	1.121	1.528	1.106	1.453
27	LGAU	0.917	1.541	1.259	2.116	1.208	1.832
29	*LDOF	0.555	1.095	1.087	2.144	1.054	1.594
30	LGEM	0.828	1.154	0.959	1.337	0.964	1.290
34	NRES	0.437	0.850	0.735	1.429	0.829	1.242
35	SRUF	0.692	1.628	1.270	2.990	1.188	2.125
36	TBAT	1.110	1.497	1.213	1.635	1.199	1.585
37	TMIN	1.082	1.768	1.449	2.367	1.369	2.055
			GONOSTOMATIDAE				
38	BPED	1.003	1.660	1.297	2.146	1.249	1.905
39	*IOVA	1.312	1.656	1.391	1.756	1.374	1.718
41	VTRI	1.233	1.651	1.583	2.119	1.488	1.905
42	*VATT	1.022	1.381	1.174	1.586	1.155	1.514
43	*VPOW	0.769	1.342	1.032	1.801	1.026	1.599
			STERNOPTYCHIDAE				
44	AACU	1.000	1.336	1.097	1.466	1.090	1.426
45	*AHEM	1.082	1.271	1.132	1.331	1.127	1.317
46	SDIA	1.132	1.421	1.203	1.511	1.193	1.483
			MELAMPHAIDAE				
47	MPUM	1.356	1.751	1.606	2.075	1.537	1.932
48	MTYP	1.018	1.332	1.103	1.442	1.096	1.409
49	SOPI	0.857	1.298	1.021	1.546	1.018	1.459
51	PCAP	0.983	1.292	1.071	1.407	1.066	1.375
			ANOPLOGASTERIDAE				
50	ACOR	0.396	0.655	0.403	0.666	0.416	0.675
			OPISTHOPROCTIDAE				
55	RNAT	0.677	1.698	0.885	2.217	0.903	1.923

lines 21 with slopes $q = m$ and intercepts $a_* = \ln k_*$ are shown in Fig. 5. Intersections of the lines with the vertical axis $x'_* = 0$ define the limiting R'_* values k_* which are approached as $l'_* = l'/l_{max} \rightarrow 1$. Lines for the nonregressive species are approximately parallel with slopes distributed about a mean of 3/2, as expected, qualitatively, from our theoretical model. Allometric law lines for the two regressive species have slopes of the order of $-2/3$, so they are approximately orthogonal to the lines for the nonregressive species.

Parenthetically, we note that $a = \ln k$ and m for the 36 nonregressive species are inversely related with a large negative correlation coefficient, -0.96, as is to be expected when l_0 is

TABLE 6. Constants specifying the dimensional forms of Huxley's allometric law, Eqs. 16 and 18, and the dimensionless forms of the law, Eqs. 21 and 23, for the 38 Ocean Acre swimbladdered fish species in Table 1 (major axis analysis).

Species no.	Name	k	m	$k_* \times 10^2$
		MYCTOPHIDAE		
1	BSUB	$7.728(10^{-4})$	2.284	5.062
2	BIND	$3.536(10^{-4})$	2.378	5.584
5	CWAR	$2.037(10^{-2})$	1.033	2.336
7	DMET	$1.142(10^{-2})$	1.351	5.192
10	*DRAF	$1.299(10^{-2})$	1.322	5.279
11	DATL	$9.376(10^{-4})$	2.329	4.822
12	*HBEN	$2.361(10^{-2})$	1.129	3.852
13	*HHYG	$4.020(10^{-3})$	1.517	3.246
14	HTAA	$6.926(10^{-3})$	1.424	3.297
18	LURO	$2.376(10^{-3})$	1.597	3.212
19	LALA	$4.003(10^{-5})$	2.765	4.155
20	†LATE	$1.039(10^{1})$	−0.858	0.210
21	*LCRO	$1.106(10^{-2})$	1.212	3.290
22	†LCUP	3.632	−0.669	0.298
23	LFES	$4.054(10^{-3})$	1.474	2.851
25	LPHO	$5.394(10^{-3})$	1.420	3.097
26	*LPUS	$1.021(10^{-2})$	1.305	3.145
27	LGAU	$3.547(10^{-2})$	1.608	3.434
29	*LDOF	$5.666(10^{-3})$	1.493	3.594
30	LGEM	$2.086(10^{-2})$	1.130	3.800
34	NRES	$1.853(10^{-2})$	1.024	2.051
35	SRUF	$9.533(10^{-4})$	1.853	4.263
36	TBAT	$7.058(10^{-3})$	1.403	3.821
37	TMIN	$1.460(10^{-3})$	1.822	3.892
		GONOSTOMATIDAE		
38	BPED	$3.085(10^{-3})$	1.644	4.269
39	*IOVA	$7.410(10^{-3})$	1.559	5.398
41	VTRI	$3.341(10^{-3})$	1.821	5.384
42	*VATT	$9.374(10^{-3})$	1.360	3.415
43	*VPOW	$1.020(10^{-2})$	1.348	3.475
		STERNOPTYCHIDAE		
44	AACU	$2.153(10^{-2})$	1.265	6.243
45	*AHEM	$2.388(10^{-2})$	1.227	5.303
46	SDIA	$1.902(10^{-2})$	1.346	6.503
		MELAMPHAIDAE		
47	MPUM	$3.391(10^{-3})$	1.818	4.151
48	MTYP	$1.526(10^{-2})$	1.258	4.584
49	SOPI	$1.459(10^{-2})$	1.250	3.639
51	PCAP	$1.412(10^{-2})$	1.225	3.979
		ANOPLOGASTERIDAE		
50	ACOR	$1.484(10^{-1})$	0.527	1.853
		OPISTHOPROCTIDAE		
55	RNAT	$8.637(10^{-3})$	1.357	3.548

small compared with the length ranges involved (Lumer et al. 1942). There is little physical significance in this correlation, however, since k represents nonexistent values of R'/l_0 at $l = l_0 = 1$ mm. The correlation coefficient between $a_* = \ln k_*$ and m is independent of l_0 and is quite different, viz., 0.46.

Histogram distributions illustrating the interspecific properties of m and k_* for the 36 nonregressive species group are

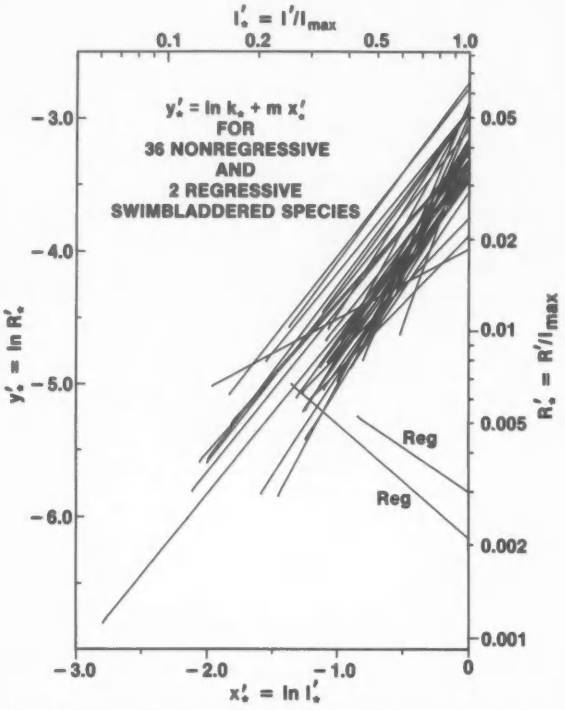

FIG. 5. Dimensionless allometric law structural relation lines for the 38 Ocean Acre swimbladdered species. The constants $a_* = \ln k_*$ and m were obtained from major axis analysis (see Table 6).

shown in Fig. 6. Using the χ^2-test for normality described above, both distributions were found to be lognormal at the 5% level. The curves in Fig. 6a and b are lognormal distributions computed from the means and variances of $\ln m$ and $a_* = \ln k_*$, respectively, and scaled to match the histogram ordinates. The ranges of m and k_* for the 22 myctophid species, viz., $1.024 \leq m \leq 2.765$ and $2.051 \leq k_* \times 10^2 \leq 5.584$, encompass most of the m, k_* values for the other 14 species. Only *A. cornuta* (50) from the family Anoplogasteridae has a lower value of m, and also a lower value of k_*. The only species with k_* values larger than the myctophids are the sternoptychids *Argyropelecus aculeatus* (44) and *Sternoptyx diaphana* (46). For the 12 gonostomatid, sternoptychid and melamphaid species, m values fall between 1.225 and 1.821, the lower portion of the myctophid m range. Corresponding k_* values for the 12 species fall for the most part within the upper k_* range for the myctophids.

From the diversity of swimbladder structure in mesopelagic species (Marshall 1960), one might expect differences in specific growth trends. That the observed differences are physically significant in some part, and not due solely to sampling fluctuations, is suggested by the specific variability of the confidence intervals for m in Table 5, some of which are disjoint. Using $\bar{m} = 3/2$ and $\bar{k}_* = 0.04$, we may nominally characterize the swimbladder growth trend in the nonregressive species group with the structural relation $R'_* = 0.04\,(l'_*)^{3/2}$.

Estimates of θ *in the theoretical structural relation model* — The parameter θ in 30a can be estimated for certain of our species with the help of fish displacement volume measure-

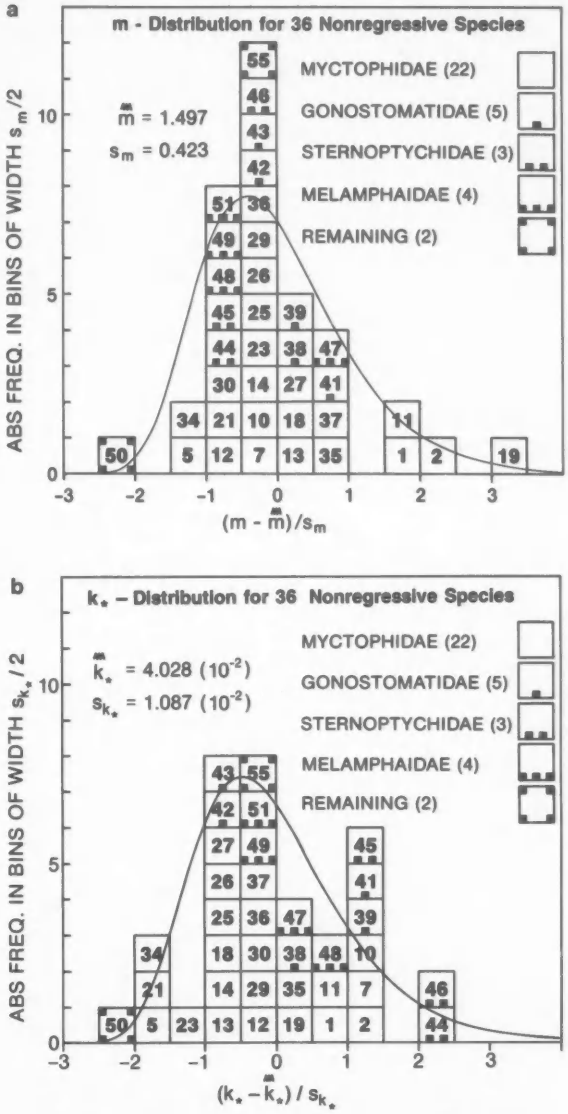

FIG. 6. Histogram plots of the dimensionless allometric law constants m in (a) and k_* in (b) for the 36 nonregressive swimbladdered species group. Species associated with each range increment of m and k_* are identified by their respective species numbers in Table 1. Membership in the four principal families represented is also indicated. Both m and k_* are found to be lognormally distributed. The curves are scaled log-normal distributions with parameters derived from the data.

ments reported by Kleckner and Gibbs (1972). The available $x = \ln(l/l_0)$ and $z = \ln(V_F/V_0)$ data for each species were assumed bivariate normal and the slope m_1 of the major axis was used to estimate q_1 in 29. Point estimates $q_1 = m_1$ and $q = m$ were used to calculate θ from 30b. Individual θ values in Table 7 are seen to be close to 1, as postulated in the model. For these eight species, $\bar{\theta} \pm s_\theta = 0.864 \pm 0.145$. If θ were exactly equal to 1 for each species, $q = m$ and $q_1/2 = m_1/2$ would be equal. Differences between the 95% confidence intervals for $m_1/2$ in Table 7 and those for m in Table 5 for these species (in particular, the fact that these intervals are disjoint for **Hygophum benoiti* (12) and **Lampanyctus crocodilus* (21)), suggest that $m_1/2$ and m are not generally equal, and that the completely idealized form of our hypothesis, viz., $\theta = 1$, is unfounded. Departures of θ from 1 are not entirely due to sampling variability, but again reflect the existence of specific differences in swimbladder growth trends.

The mean $\bar{\theta}$ for the 36 nonregressive species group may be estimated with some simple assumptions. Taking the mean of both sides of Eq. 30b, and assuming that θ is statistically independent of q_1, so that $\widetilde{\theta q_1}$ may be replaced by $\bar{\theta} \cdot \bar{q}_1$, we obtain $\bar{\theta} = 2\bar{q}/\bar{q}_1$. From the fact that $m_1 \pm s_{m_1} = 3.094 \pm 0.272$ for the eight species, and from general considerations, it seems reasonable to assume $\bar{q}_1 \simeq 3$. From measurement, $\bar{q} = \bar{m} = 3/2$, so $\bar{\theta} \simeq 1$, indicating that, in an average sense, swimbladder wall area S_B grows in proportion to fish volume V_F or mass M for the 36 nonregressive species group.

Variability in Percentage Swimbladder Volume and Fish Buoyancy

To keep from sinking, a negatively buoyant mesopelagic fish must maintain some minimum level of swimming activity in order to generate a dynamic lift force equal to its weight ΔW in water. In swimbladdered fish, the buoyancy provided by a gas-filled swimbladder reduces ΔW and therefore the energy expenditure required to maintain depth (Denton and Marshall 1958; Alexander 1966, 1972). However, maintaining a gas-filled swimbladder for this purpose also requires expenditure of energy, and some species find it advantageous to supplement, or even replace, buoyancy contributions from the swimbladder by chemical means, reducing their average tissue density by storing low density lipids and adjusting water content (Capen 1967; Butler and Pearcy 1972; Neighbors and Nafpaktitis 1982). We now investigate the buoyancy properties of some of the species in Table 7.

The neutral buoyancy condition for a fish with a swimbladder gas volume V_G and average tissue density ρ_t in water of density ρ_w is often written as

$$(31) \quad V_G/V_F = (\rho_t - \rho_w)/\rho_t.$$

This approximate relation, sufficient for present purposes, presupposes relatively shallow depths so that swimbladder gas density ρ_G is small enough to be neglected in comparison with ρ_t (see, e.g., Capen 1967). Water weight ΔW expressed as a fraction of weight W in air is given approximately by

$$(32) \quad \Delta W/W = (\rho_t - \rho_w)/\rho_t - (V_G/V_F)$$

which is zero when the neutral buoyancy condition 31 is satisfied. For qualitative estimates of the size of a gas-filled swimbladder needed to provide neutral buoyancy, it is common to use Taylor's (1922) value $\rho_t = 1.076$ g/cm^3 for fat-free fish tissue and $\rho_w = 1.026$ g/cm^3 for sea water, in which case V_G/V_F must be 0.046, or about 5% (Jones and Marshall 1953).

For simplicity, we assume that the nonregressive swimbladdered species considered below have thin-walled, gas-filled swimbladders, so that V_G/V_F may be replaced by $V_B/V_F = B_*$. The neutral buoyancy condition 31 then becomes

$$(33) \quad B_* = (\rho_t - \rho_w)/\rho_t.$$

Although values of ρ_t are not available to directly check this

TABLE 7. Statistical quantities calculated for 8 swimbladdered species using (l,V_F) data of Kleckner and Gibbs (1972), where $x = \ln(l/l_0)$, $z = \ln(V_F/V_0)$ and $V_0 = 1\ cm^3$. The orthogonal regression coefficient m_1 is used to estimate the allometric exponent q_1 in Eq. 29. The number n of data points and the correlation coefficient r_{xz} are followed by the lower 95% confidence limit $m_{11}/2$, the point estimate $m_1/2$, the upper 95% confidence limit $m_{12}/2$, θ, and the intercept $(\ln k_1)_R$ and slope b_{zx} of the z-on-x regression line. Major axis and reduced major axis estimates of q_1 for each species are nearly the same as the regression estimate b_{zx}, since the correlation coefficients r_{xz} are very close to 1.

Species no.	Name	n	r_{xz}	$m_{11}/2$	$m_1/2$	$m_{12}/2$	$\theta = 2m/m_1$	$(\ln k_1)_R$	b_{zx}
10	*DRAF	7	0.9945	1.442	1.618	1.838	0.817	−11.82	3.204
12	*HBEN	7	0.9907	1.320	1.532	1.816	0.737	−11.16	3.013
13	*HHYG	4	0.9864	0.822	1.284	2.514	1.181	− 9.09	2.508
21	*LCRO	8	0.9957	1.506	1.647	1.815	0.736	−12.95	3.268
26	*LPUS	6	0.9949	1.334	1.524	1.771	0.856	−11.82	3.021
29	*LDOF	6	0.9909	1.241	1.481	1.818	1.088	−10.73	2.913
42	*VATT	4	0.9975	1.465	1.786	2.268	0.761	−13.44	3.555
45	*AHEM	4	0.9921	1.070	1.505	2.411	0.815	−10.25	2.967

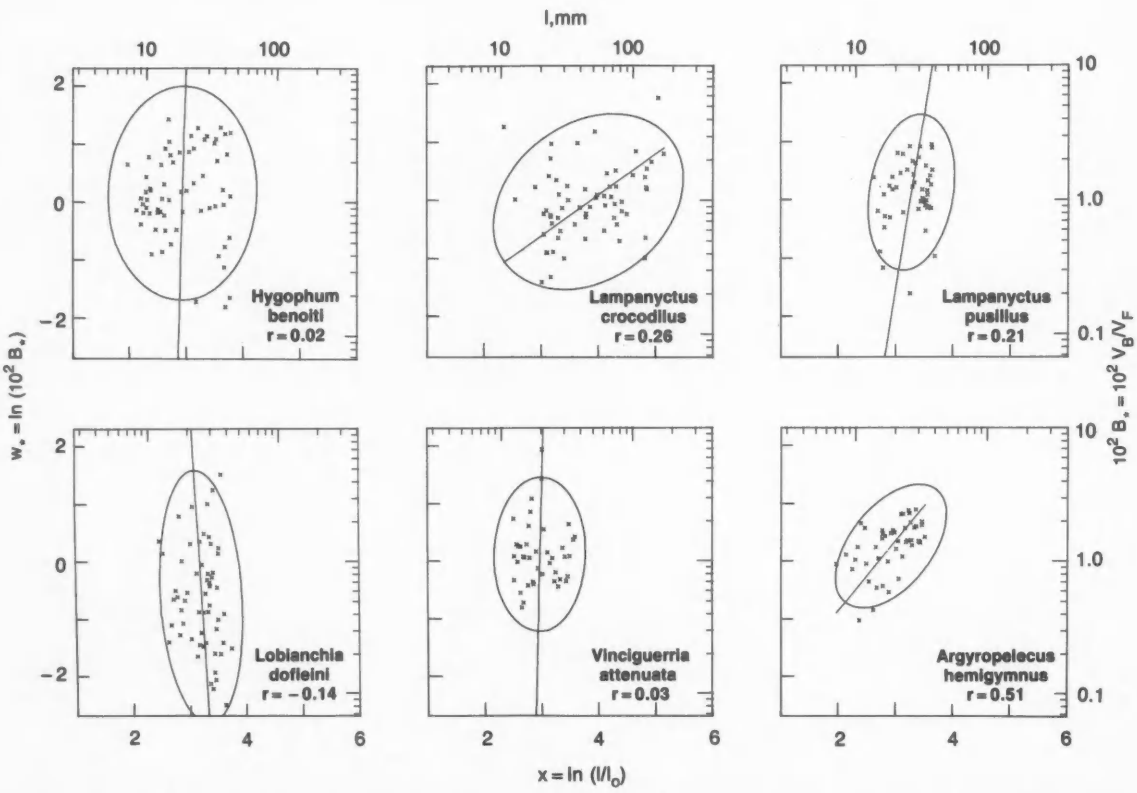

FIG. 7. Standard length-percentage swimbladder volume distributions for six swimbladdered fish species. With the possible exception of **Hygophum benoiti* (12), the (x,w_*)-distributions for these species are found to be bivariate normal. The major axis and 95% equiprobability ellipse are drawn for each distribution. Linear trends are ill-defined due to the low absolute values of the correlation coefficient r. Swimbladder contributions to positive buoyancy are highly variable and independent of fish length except for the sternoptychid **A. hemigymnus* (45) (see text).

relation, considerable insight into specific buoyancy properties may be obtained by other means. Thus, it is shown below that B_* is a random variable with a well-defined probability density function. Let $(B_*)_p$ represent the p-percent point of a given B_* distribution for arbitrary p. By definition, p-percent of the individuals in the population have values $B_* \leq (B_*)_p$. To be neutrally buoyant, their (B_*,ρ_t) values must satisfy 33. A necessary condition for this to be true is that the tissue densities ρ_t of these individuals are less than, or at most equal to, $(\rho_t)_p$, the tissue density corresponding to $B_* = (B_*)_p$ in 33. Now, if for a particular choice of p, $(\rho_t)_p$ is too near ρ_w to be physically realizable, then at least p-percent of the population must be negatively buoyant. (The qualification "at least" is necessary because a portion of the population for which $B_* > (B_*)_p$ may

TABLE 8. Bivariate normal distribution parameters and regression coefficients for the (x,w_*)-scattergrams of six nonregressive swimbladdered fish species in Fig. 7. Marginal probability density functions of 10^2B_* are lognormal with parameters $(\overline{w}_*, s^2_{w*})$. Percentage points of these probability densities are given for $p = 50$ and 90% together with the corresponding tissue densities computed from the ideal buoyancy condition Eq. 33 for fish with gas–filled swimbladders of negligible wall thickness.

	Hygophum benoiti (12)	*Lampanyctus crocodilus* (21)	*Lampanyctus pusillus* (26)	*Lobianchia dofleini* (29)	*Vinciguerria attenuata* (42)	*Argyropelecus hemigymnus* (45)
$\bar{x}$	2.936	3.827	3.280	3.218	2.980	2.929
$\bar{w}_*$	0.1525	−0.0266	0.1281	−0.6204	0.1217	0.2636
s_x	0.5345	0.6773	0.3082	0.2981	0.3282	0.3963
s_{w_*}	0.7521	0.6164	0.5461	0.9032	0.5457	0.4368
r	0.0197	0.2622	0.2060	−0.1445	0.0292	0.5105
b	0.0277	0.2386	0.3649	−0.4378	0.0485	0.5627
m	35.46	0.7032	6.029	−18.73	36.42	1.209
g	1.407	0.9102	1.772	−3.029	1.663	1.102
$(10^2B_*)_{50}$	1.2	1.0	1.1	0.5	1.1	1.3
$(\rho_t)_{50}$	1.038	1.036	1.038	1.032	1.038	1.040
$(10^2B_*)_{90}$	3.1	2.1	2.3	1.7	2.3	2.3
$(\rho_t)_{90}$	1.058	1.048	1.050	1.044	1.050	1.050

be negatively buoyant, and also because we are using B_* in place of V_G/V_F in our neutral buoyancy criterion 33.) This result is used below. One general point should be noted. Fish buoyancy is inherently a stochastic property. Consequently, buoyancy classification of an individual species on the basis of measurements on just a few specimens will not usually be statistically significant, regardless of measurement accuracy.

Plots of $w_* = \ln(10^2B_*)$ vs x for 6 of the 8 species in Table 7 are shown in Fig. 7. Since only a few measurements of V_F are available for these species (see Table 7), regression equation values for $z = \ln(V_F/V_0)$ were necessarily employed in computing w_*. Errors in w_* arising from the use of regression, rather than measured, values of z are not judged serious, since r_{xz} is so close to 1 for these species that prediction errors in z must be small. The (x,w_*)-distributions for all species except *Hygophum benoiti* (12) are found to be bivariate normal, having passed all four statistical tests. The *H. benoiti* (12) distribution is also considered bivariate normal here, since its x and d test failures were only "almost significant." Distribution parameters for the 6 species are given in Table 8.

Especially notable is the statistical independence of w_* and x for the four myctophid species *Hygophum benoiti* (12), *Lampanyctus crocodilus* (21), *Lampanyctus pusillus* (26), *Lobianchia dofleini* (29) and the gonostomatid *Vinciguerria attenuata* (42). At the 5% level, there is no significant departure from the hypothesis that the population correlation coefficients are zero. (Indeed, the virtual absence of linear trends of w_* with x revealed by visual inspection suggests that slope estimates b, m, and g of supposed linear structural relations between w'_* and x^1_* are not physically meaningful.) For these five species the conditional and marginal 10^2B_* probability density functions (pdfs) of the respective parent populations may be considered the same. This is not true, however, for the sternoptychid *Argyropelecus hemigymnus* (45), since $r = 0.51$ represents a highly significant deviation from the hypothesis of a zero population correlation coefficient at the 5% level. Fifty and ninety percent points $(10^2B_*)_p$ of the marginal lognormal pdfs $\lambda(10^2B_*; \overline{w}_*, s^2_{w*})$ are found in Table 8, along with corresponding values of $(\rho_t)_p$.

The range of $(\rho_t)_{90}$ for the six species is 1.044 to 1.058 g/cm^3 and that of $(\rho_t)_{50}$, 1.032 to 1.040 g/cm^3. It is problematic whether such low tissue densities actually occur in these nonregressive species. Assuming lipids of average density 0.9 g/cm^3 are stored to reduce ρ_t, and taking the average tissue density of the remaining portion of the fish to be 1.076 g/cm^3, then the lipid content corresponding to the range of $(\rho_t)_{90}$ values is 8.7–15.7% of total fish wet weight. The corresponding lipid content for the range of $(\rho_t)_{50}$ values is 17.7–21.8%. Bone (1973) found lipid contents in specimens of *Myctophum punctatum* dipnetted in the Bay of Biscay to be 2.0–2.6% of total wet weight. His measurements of V_G and V_F on fresh specimens with noncontracted swimbladders gave $10^2(V_G/V_F)$ values from about 0.1 to 4.7%, a range which includes nearly all our 10^2B_* values for the six species (Fig. 7). Average lipid contents found by Neighbors and Nafpaktitis (1982) for two other of our nonregressive species, *Notoscopelus resplendens* (34) and *Taaningichthys bathyphilus* (36), were 5.6 and 3.8%, respectively. Assuming these lipid contents are representative of the six species, we infer that well over 90% of each population must be negatively buoyant. Such estimates are necessarily somewhat crude, since we do not have reliable values for the minimum tissue densities of the six species. The property of negative buoyancy inferred for these species was found experimentally for *Myctophum punctatum* by Bone (1973). Most of his dipnetted specimens sank in surface sea water, and presumably were negatively buoyant at other depths below the surface as well.

Assuming lipid content is small, we expect ρ_t to increase with fish length, on the average, as found for *M. punctatum* by Bone (1973). For the five species in which the distribution of B_*, and hence, by assumption, also that of V_G/V_F, remains the same as length increases, we see from 32 that $\Delta W/W$ must increase, on the average, with increasing fish size. These five species, particularly the myctophids, are diel vertical migrators, and it is apparently not advantageous for these species to maintain larger swimbladders to offset the average increase in $\Delta W/W$ because of the physiological effort involved. In the sternoptychid *A. *hemigymnus* (45), a weak migrator, V_G/V_F increases with length on the average, lessening the average increase in $\Delta W/W$. The elaborate physical structure of *A. *hemigynmus* (45), quite different from the five other fusiform species, suggests that it may have relatively larger tissue density, and hence require more buoyant support from the swimbladder to keep $\Delta W/W$ within tolerable bounds as it grows.

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Production of Juvenile Atlantic Salmon (*Salmo salar*) in Two Acidic Rivers of Nova Scotia

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Densities, growth, and production of juvenile Atlantic salmon (*Salmo salar*) were estimated at sites in two acidic rivers of different pH, the Westfield and North rivers, in Nova Scotia, Canada, from 1982 to 1984. Annual production was significantly lower in the more acidic Westfield River (<0.4 $g \cdot m^{-2} \cdot yr^{-1}$ at pH 4.7–5.4) than in the North River (1.4 $g \cdot m^{-2} \cdot yr^{-1}$ at pH 5.6–6.3). Low production rates in the more acidic river were attributable to the lower densities and poorer survival of juveniles at the lower pH levels. Low densities of parr in the more acidic river were a function of the high initial mortality of postemergent fry, and the mortality of parr during periods of pH minima such as over winter. Marking and recapture information and trapping at a weir for counting migrating fishes indicated that there was little emigration of parr other than in the spring when some movements occurred preceding and during smoltification. Average potential yield of 2-yr smolts was 0.8 smolt/100 m^2 in the Westfield River and in the range of 2.7–6.6 smolts/100 m^2 at higher pH levels in the North River. Growth was not limiting to production at the lower pH levels. After the first winter, 1-yr-old parr were much larger at the lower pH than they were at less acidic pH levels, the faster growth possibly resulting from the lower density of parr at the lowest pH. A temperature-related biphasic growth of 1-yr-old parr with periods of maximum growth in spring and autumn was recorded. Reduced production, a result of deleterious effects of low pH on survival and density in the Westfield River was probably sufficient to prevent the continued maintenance of a naturally produced salmon population in that river, whereas production at higher pH levels in the North River was comparable to that in near-neutral waters of other areas.

On a mesuré de 1982 à 1984 les densités, la croissance et la production de jeunes saumons de l'Atlantique (*Salmo salar*) en des points de deux rivières acidifiées à pH différent, la Westfield et la North, en Nouvelle-Ecosse, au Canada. La production annuelle était nettement plus basse dans la Westfield, rivière aux eaux plus acides (<0,4 $g \cdot m^{-2} \cdot an^{-1}$ avec un pH de 4,7–5,4) que dans la North (1,4 $g \cdot m^{-2} \cdot an^{-1}$ avec un pH de 5,6–6,3). Le faible taux de production dans la rivière plus acidifiée était attribuable à la densité plus basse et au taux de survie plus faible des juvéniles au pH plus bas. Les densités plus faibles de tacons étaient fonction de la forte mortalité initiale des alevins après l'émergence, et de la mortalité des tacons pendant les périodes de pH très bas, notamment pendant l'hiver. Les données obtenues par marquage et recapture et par dénombrement à une barrière établie pour compter les migrateurs ont indiqué une faible émigration des tacons, mis à part au printemps où on observait un peu de mouvement avant et pendant la smoltification. Le rendement moyen potentiel en smolts de deux ans était de 0,8 smolts/100 m^2 dans la Westfield et se situait dans la fourchette de 2,7–6,6 smolts/100 m^2 dans les eaux de la North au pH supérieur. La croissance ne limitait pas la production au pH inférieur. Après le premier hiver, les tacons d'un an étaient beaucoup plus gros au pH bas que dans les eaux moins acides, ce rythme de croissance plus rapide pouvant s'expliquer par la moindre densité des tacons dans les eaux plus acides. On a enregistré une croissance biphasée liée à la température chez les tacons d'un an, avec des maximums de croissance au printemps et à l'automne. La réduction de la production, résultat des effets néfastes du faible pH sur la survie et la densité dans la Westfield, était probablement suffisante pour empêcher le maintien d'une population naturelle dans cette rivière, alors que la production dans la rivière North, au pH plus haut, était comparable à celle des eaux presque neutres dans d'autres régions.

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Acidification of surface waters in Nova Scotia, Canada, as a result of acid deposition from long-range sources in addition to the effects of organic acids from peatland and bogs in river catchments (Gorham et al. 1986; Kerekes et al. 1986; Underwood et al. 1987) has been associated with significant losses of Atlantic salmon (*Salmo salar*) in rivers of the southwest area of that province (Watt et al. 1983). In these rivers, significant mortalities of salmon eggs, fry, and parr have been identified in relation to low pH levels, and the lethal effects have been attributed to high H^+ concentrations at low environmental calcium concentrations (Lacroix 1985; Lacroix et al. 1985; Lacroix and Townsend 1987). The potential toxicity of metals such as aluminum is considered as minimal because of the extensive chelating capacity of organic acids in these waters rich in organic matter (Lacroix and Kan 1986).

The North Atlantic Salmon Conservation Organization (NASCO) recently indicated that questions relating to the influence of acidification of freshwater habitat on growth and sur-

vival of juvenile salmon, the implications for smolt production, and assessment of the loss of salmon production in areas affected by acid rain were of prime importance (ICES 1988). Of the factors now recognized as influencing production of juvenile salmon by affecting growth rate or population density, the effects of the chemical composition of the water on growth and survival of salmon are little understood (Egglishaw and Shackley 1985), yet it is a subject which has become particularly relevant in view of the acidification of salmon rivers through acid rain (Watt et al. 1983).

Studies of salmon density, growth, and production are relatively rare, and of these only one to date has examined differences in production, growth, and food of juvenile salmon on the basis of the differences in acidification between two rivers (Fjellheim et al. 1987). In a recent study, the structure of fish communities in three Nova Scotia rivers of differing pH was examined in relation to acidity (Lacroix 1987b). Juvenile salmon densities and biomass were increasingly lower in more acidic water and were, therefore, apparently related to pH in these rivers, indicating that a detailed examination of growth and survival could possibly yield answers to some of the questions posed in relation to the effects of acidification on production of juvenile salmon. This study, therefore, examined and compared the density and growth of juveniles and their effects on freshwater production of Atlantic salmon, as both total elaboration of fish tissue per year and potential yield of smolts, in two rivers of the southwest area of Nova Scotia were differences in chemical compositions are reflected mainly as differences in pH.

Materials and Methods

Density and biomass estimates of juvenile salmon were made at sites in the Westfield River and the North River in Nova Scotia on 12 occasions between June 1982 and June 1984 (Fig. 1). In the Westfield River, five sites were sampled during 1982 and three sites during 1983–84 to describe the salmon population, whereas one site was sampled in the North River from 1982 to 1984. The habitat sampled included at least one pool–riffle stretch in each site, and it contained spawning and nursery areas for salmon based on observations made in 1982 in both rivers.

The Westfield River has a drainage area of about 142 km^2, and had a mean water discharge of 3.2 $m^3 \cdot s^{-1}$ over the 2 yr of study, and the North River, 193 km^2 and 4.8 $m^3 \cdot s^{-1}$, respectively. Water flow was recorded continuously at a gauging station on the Westfield River and was estiamted in the North River from a rating for a station downstream on the LaHave River. Mean monthly discharge was only slightly less in the Westfield River than in the North River, and the seasonal trends were identical (Fig. 2). The North River is the larger of the two rivers, but the study site in that river was a channel where the river is divided by an island. The mean channel width was 7.6 m in the North River and 11.9 m in the Westfield River. Mean water depth was slightly lower in the Westfield River (20 cm compared with 24 cm in the North River) and mean water velocity was higher (0.14 $m \cdot s^{-1}$ compared with 0.08 $m \cdot s^{-1}$ in the North River), but the differences among sites were not significant (ANOVA, $P>0.05$). The average gradient, determined using a transit in river reaches with study sites, was 1.23% in the Westfield River and 1.29% in the North River. Depth distribution profiles were similar in both rivers, and that for the North River was within the range for sites in the Westfield River (Fig. 3A). The Westfield River had a greater maximum depth, but in both rivers about 90% of the area was less than 40 cm deep at times of average water flow. The mean grain size of bed materials was 51 mm in the Westfield River and 58 mm in the North River (grid-by-number method of Kellerhals and Bray 1971), and there was no significant difference among sites (ANOVA, $P>0.05$). The bed materials were slightly finer in the Westfield River than in the North River, which had more cobble (64–256 mm) and a few boulders (>256 mm). About 80% of the bed materials were finer than 64 mm (i.e., consisted of pebble and gravel) in the Westfield River compared with about 50% in the North River, but about 30% was finer than 32 mm in both rivers (Fig. 3B). Although there were slight differences, the overall physical characteristics of the habitat selected for study areas were similar in both rivers.

Annual counts of Atlantic salmon redds were made in the Westfield and North rivers in sampling sites and adjacent areas within 100–200 m to determine spawning density. Egg deposition in these areas was estimated as described by Lacroix (1987a), using the ratio of 1-sea-winter to multi-sea-winter salmon (1SW:MSW) in each river and the average fecundity of these small and large salmon for southwest Nova Scotia. The data for the North River are from the Morgan Falls fishway downstream on the LaHave River (Fisheries and Oceans Canada, Freshwater and Anadromous Division, Halifax, unpublished data) and those for the Westfield River are from a weir for counting migrating fishes operated in 1981–83 in that river. The density of juvenile salmon was estimated using the sequential-removal method. Fish were captured from sites (range 500–1500 m^2) enclosed with barrier nets (seines of 5 mm delta-style netting with a bottom 0.5 m wide burlap apron covered with rocks) by electrofishing using 450–650 V pulsed direct current (Smith-Root Type VIII-A and Type XI backpack electrofishers for water of low conductivity). The method is described by Gray et al. (1978). Fish were removed by shocking from the upstream to downstream net, keeping voltage and effort constant, until the number of fish caught declined significantly (usually 3–6 successive fishings). A standardized period of 1 h was allowed for uncaught fish to recover between successive fishings. During this period the captured fish were anesthetized with tricaine methanesulphonate (Syndel Laboratories Ltd.), and counted, measured to the nearest millimetre (fork length), and weighed (to the nearest 0.1 g, or the nearest 0.01 g for subsamples of young fish weighing <1.0 g). All parr captured were marked, either by clipping part of a pelvic fin or by an integumentary brand made using a hot wire, and they were retained in shaded holding cages in the stream beyond the site and returned alive to the site after the final collection. The number of marked fish recaptured on successive sampling dates provided an estimate of the proportion of resident parr. The technique was also used to examine the movements of juvenile salmon among sites in the Westfield River. Age was estimated by the number of apparent annuli on flank scales and by independent inspection of polymodal frequencies of body length. All age determinations by scales were based on at least two independent readings by different observers.

Density estimates of salmon parr were calculated using the maximum likelihood solution to the removal method (Zippin 1956; Seber 1973) and catch depletion data for separate age-classes. Estimates were calculated separately by age-class because the probability of capture is size-dependent (Vibert 1967; Randall and Paim 1982). The sampling variance for individual estimates was calculated as described by Zippin (1956),

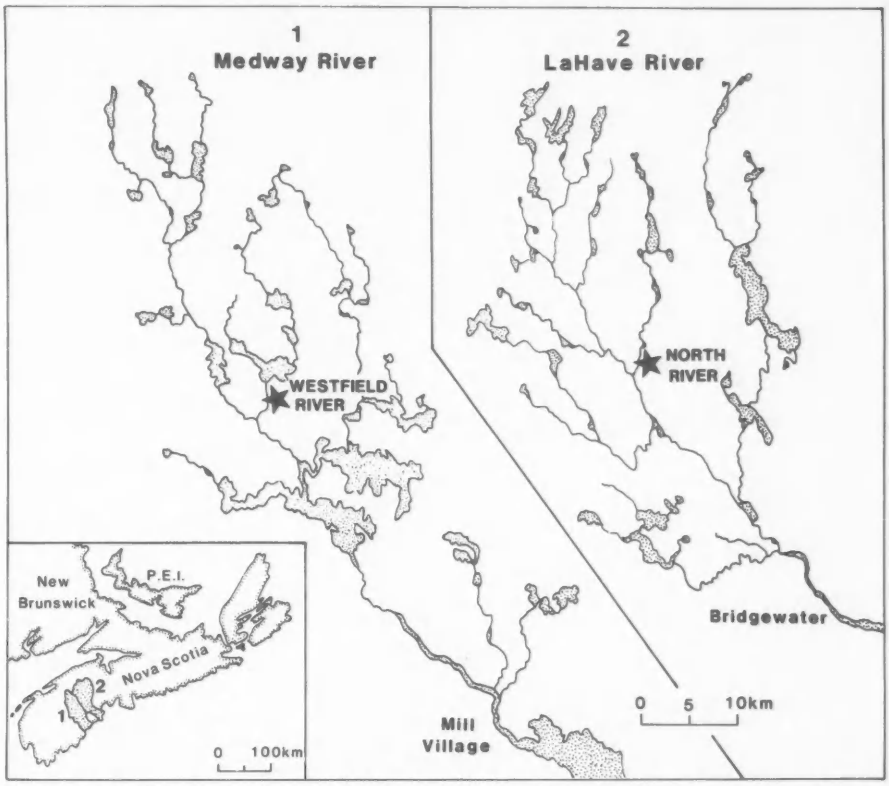

FIG. 1. Westfield River in the Medway River watershed and the North River in the LaHave River watershed, and sections in which fish density and production were estimated.

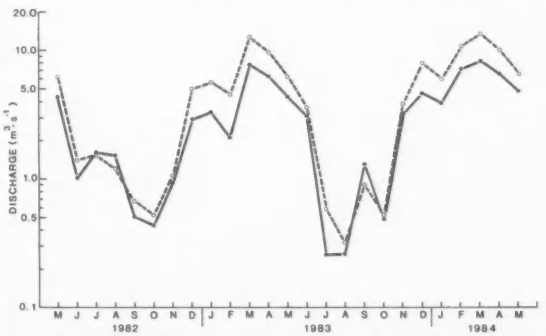

FIG. 2. Mean monthly water discharge measured in the Westfield River (●) and estimated for the North River (○). Discharge data from Environment Canada, Water Resources Branch are from station no. 01EE004 on the Westfield River and station no. 01EF001 on the LaHave River proportionally rated for the North River.

but for an average estimate combining sites in the Westfield River, a sampling variance incorporating both total and measurement variances was derived based on equations described by Bohlin (1981). In cases where the method of estimation failed (e.g., infinite estimates), or when the catchability was too low and considered inaccurate (<0.5), total catch was substituted for the estimate of density (Zippin 1956; Carle and Strub 1978). For small populations and catchability <0.5, Bohlin (1982) suggested using a catchability estimate derived from a pooled population from several occasions. However, this assumes that catchability is constant, which can lead to errors in data interpretation (Schnute 1983). Because of the scarcity of juvenile salmon in the more acidic river, the total catch was considered a more accurate estimate of density when catchability could not be accurately determined. In such cases, total catch was small (usually <5) over large areas and no fish were caught in successive attempts (2–5) after the first fishing. The goodness-of-fit statistic for a multinomial distribution where the population size is not known (Seber 1973) was calculated for density estimates, a good fit indicating that the probability of capture remained constant throughout an estimate. For 0+ salmon, the most abundant age-class, 88% of density estimates had an excellent fit (P <0.05).

Growth was compared using the mean length and weight in successive samples. Additional samples were also taken on several occasions between density estimates to obtain growth data when intervals were long, such as over winter. Because there were no differences in mean lengths and weights among fish from the sites in the Westfield River (ANOVA, P >0.05), growth data for fish from all sites in that river were pooled to calculate mean lengths and weights.

Production (P) was calculated from mean biomass ($\bar{B}$) and instantaneous growth rate (G_w) for each age-class during a known time interval (Δt) using equations from Chapman (1978b). These were developed from exponential rates of growth (G_w) and mortality (Z, includes emigration) which are appro-

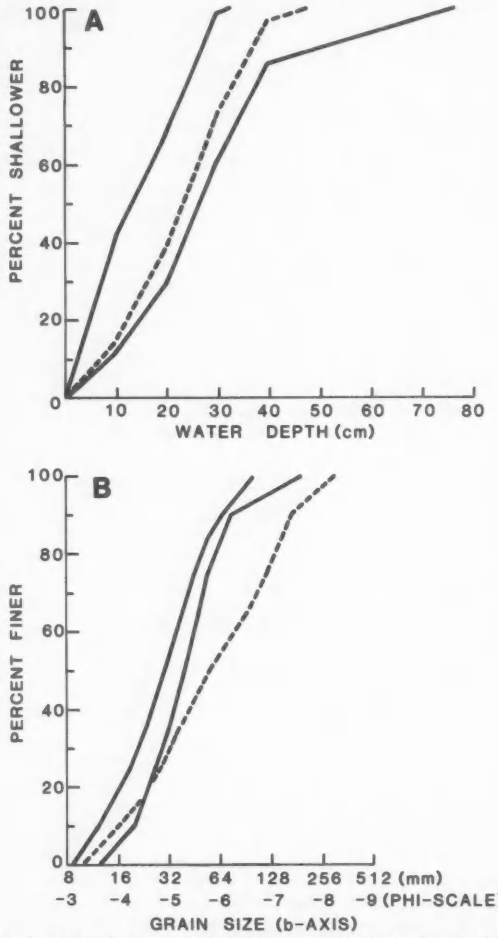

FIG. 3. Physical characteristics of the study sites in the Westfield River (range, solid lines) and site in the North River (broken line) during average water flow. (A) Water depth distribution. (B) Streambed material size distribution.

priate if calculated over small time intervals as in this study. The equations were:

$$P = G_w \bar{B} \Delta t$$

$$G_w = (\ln W_2 - \ln W_1)/\Delta t$$

$$\bar{B} = B_1[\exp(G_w - Z)\Delta t - 1]/(G_w - Z)\Delta t \quad \text{if } G_w > Z$$

$$\bar{B} = B_1\{1 - \exp[-(Z - G_w)\Delta t]\}/(Z - G_w)\Delta t \quad \text{if } G_w < Z$$

$$Z = -(\ln N_2 - \ln N_1)/\Delta t$$

where N_1, N_2 and W_1, W_2 are densities and mean weights, respectively, of an age-class at the beginning and end of the interval Δt, and B_1 is the initial biomass for the interval.

In addition to marking–recapture data, a weir installed for counting migrating fishes in the Westfield River (about 2.5 km from the mouth) was used to examine possible immigration and emigration of parr within that river. The weir, similar in design to that described in Anderson and McDonald (1978), stretched diagonally across the river from bank to bank and had two traps positioned to capture upstream and downstream migrants. Lath

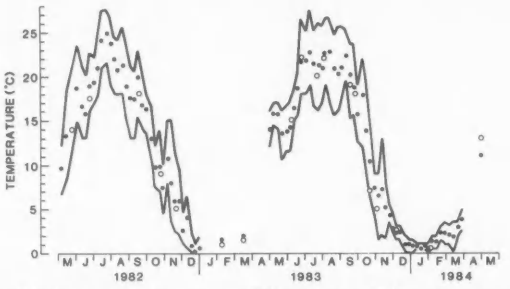

FIG. 4. Mean weekly temperature (●) and mean daily maximum and minimum temperature for each week in the Westfield River (solid lines) and occasional temperature measurements in the North River (○).

(aluminum conduit) spacing and trap design and screen mesh size were modified to increase efficiency of capture of small salmon parr and smolts. The weir and traps were operated from April 25 to December 3 in 1982 and May 3 to December 6 in 1983. They were completely removed in December because of high water flow and could only be reinstalled after spring runoff in April thus preventing a complete assessment of migrations, especially that of presmolts which may have emigrated before the smolt run. Smolts were captured from April 26 to May 29 in 1982 and from May 5 to June 7 in 1983. The data generally provide a good indication of movements during periods and at water temperatures when juvenile salmon are normally active (Rimmer et al. 1983). The weir was also used to estimate the age-frequency distribution of smolts and of returning salmon, and the proportion of salmon of hatchery origin in the escapement.

Water samples were collected in the streams at each estimate and analyzed using standard methods (APHA et al. 1976) unless otherwise noted. Water temperature was recorded continuously in the Westfield River and on sampling occasions in the North River. The pH level was measured weekly in the Westfield River, but usually only at times of fish sampling in the North River. Alkalinity (total inflection point) and acidity were determined by electrometric titration. Metals (Na^+, K^+, Ca^{2+}, Mg^{2+}) were measured by flame atomic absorption spectroscopy, chloride by mercuric nitrate titration, and sulfate by the turbidimetric method (1982) and methylthymol blue method (1983–84). Filtered (0.45 μm membrane porosity) and acidified (concentrated HCl, pH <2) samples were analyzed for dissolved organic carbon (DOC) content by the combustion-infrared method. Unfiltered, preserved (0.2% HNO_3, vol/vol) samples were analyzed for total extractable aluminum and iron by atomic absorption spectroscopy using the solvent extraction method (Environment Canada 1979).

Water temperature in the North River was normally within the weekly range for the Westfield River (Fig. 4). In both rivers, water temperature was close to freezing only for brief periods from late December to early February, and it increased continually from March through June to high maximum temperature values in July and August (at times >25°C). Temperature remained high (minimum >15°C) for a long period (about 3–4 mo) and only fell under 10°C in November. The acidity was higher in the Westfield River than in the North River, and the pH, alkalinity, and concentrations of Ca^{2+} and Mg^{2+} were lower (Table 1). The pH levels were always less than 5.5 in the more acidic Westfield River, whereas they were always greater

TABLE 1. Water chemistry of Nova Scotia rivers where fish were sampled between June 1982 and June 1984. The mean in $mg \cdot L^{-1}$ with range in parentheses is for 12 samples unless otherwise indicated.

	North River	Westfield River
pH (units)	5.9 (5.6–6.3)	4.9 (4.7–5.4)
Acidity ($CaCO_3$)	4.7 (2.0–8.3)	5.7 (4.1–7.7)
Alkalinity ($CaCO_3$)	2.5 (1.2–3.6)	0.03 (−0.5–0.5)
Na^+	2.92 (2.33–3.29)	2.48 (2.21–2.92)
K^+	0.36 (0.26–0.45)	0.31 (0.27–0.36)
Ca^{2+}	1.67 (1.25–2.06)	0.66 (0.58–0.82)
Mg^{2+}	0.65 (0.45–0.83)	0.42 (0.36–0.55)
Cl^-	4.4 (3.3–6.1)	4.0 (3.2–6.2)
SO_4^{2-}	3.4 (2.8–4.1)	3.4 (2.7–3.9)
DOC[a]	6.5 (4.8–12.0)	9.5 (7.0–15.4)
Al (total)	0.09[b]	0.19 (0.14–0.27)
Fe (total)	0.16[b]	0.21 (0.12–0.30)
Conductivity ($mS \cdot m^{-1}$)	2.80 (2.06–3.50)	2.40 (2.13–3.72)

[a] $N = 8$.
[b] $N = 1$.

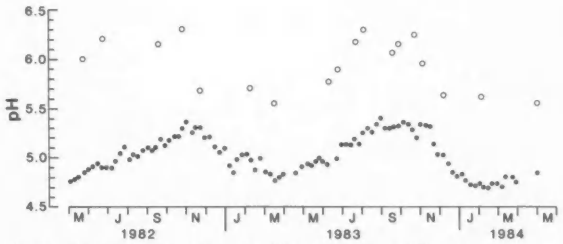

FIG. 5. Weekly pH levels in the Westfield River (●) and occasional pH measurements in the North River (○).

than 5.5 and often greater than 6.0 in summer in the North River (Fig. 5). Maximum pH levels occurred in late summer and early autumn at times of minimum water flows, whereas minimum pH levels followed the autumn rains and pH remained low throughout winter and spring. Levels of DOC and aluminum were higher in the more acidic river (Table 1).

Results

Density

Spawning took place in both rivers during November until the end of the first week of December in the years of study. The density of redds was higher in study areas of the Westfield River in years leading to the 1982 and 1983 year-classes (0.7 and 0.4 redds/100 m^2 in 1981 and 1982, respectively) than that estimated in the study site and adjacent areas of the North River (about 0.1 redds/100 m^2 in both 1981 and 1982). This resulted in an estimated deposition of 1220 and 690 eggs/100 m^2 in the Westfield River sites in 1981 and 1982, respectively, and of 190 eggs/100 m^2 in the North River over both years. It is worth noting that, in the Westfield River, 60 and 70% of the escapement counted at the weir in 1981 and 1982, respectively, were strays of hatchery origin (i.e., they had been released as smolts in other areas of the Medway River in previous years). The fry emerged from redds during the second half of the following May. Several year-classes of juvenile salmon were always present in both rivers; 0+ and 1+ fish were collected on each sampling occasion, but 2+ parr were caught infrequently. The abundance of each age-group usually decreased exponentially with time in each river and it was consistently greater in the North River than in the Westfield River (Fig. 6). Changes in density of three year-classes per unit area were followed, two of them for 2 yr and the other for 1 yr. The least-squares regressions of densities against time during the period between estimates of maximum and minimum densities (Fig.6) indicated that overall loss rates (Z), after the initial loss of fry, were similar in both rivers for each year-class (Table 2). Comparison of the slopes of the regressions for an age-class indicated no significant differences in Z between rivers (t-test, $P > 0.05$), except for the 1981 year-class the density of which increased in the Westfield River during the summer–autumn period and only declined overwinter. Correlation coefficients for these regressions were all significant at the 0.005 level (Student's t). The correlation coefficients (r in Table 2) indicate that the proportion of the variance of density attributed to its linear regression on time (r^2) was over 75% and in most cases over 90%.

Initial densities of fry after emergence and throughout June could not be accurately estimated because of small fish size and, often, high water flow. The regression equations ($\ln N = a - Zt$) were, therefore, used to estimate the numbers of fry that were potentially present in both rivers on June 1 (Table 2). These values should represent minimum initial fry densities because they do not take into account the high sensitivity of fry to low pH. The initial loss rate is, therefore, likely to have been greater than that estimated by the regression, especially at the low pH levels in the Westfield River. Nevertheless, estimated fry densities (N_f) were at least three times higher in the North River than in the Westfield River (Table 2).

The difference in density established between rivers, at the fry stage, was maintained throughout the time-course of a year-class as indicated by the least-squares regressions in Fig. 6 for the 1982 and 1983 year-classes. However, calculation of separate instantaneous loss rates for the summer–autumn and winter–spring periods indicate differences between rivers (Table 3). The summer loss of 0+ fish was greater at the higher densities in the North River, but the overwinter loss of these was more than twice as high in the Westfield River as in the North River (Table 3). In contrast, the overwinter loss rate of 1+ parr was considerably greater in the North River than in the Westfield River where few fish remained at the beginning of that interval. This loss was probably not indicative of mortality because of the high net emigration of parr as 2-yr smolts during that interval. The proportion of 2-yr smolts in the run in the Westfield River was 92 and 90% in 1982 and 1983, respectively, and it was assumed to be similar in the North River on the basis of the low proportion of 2+ and older parr in the population. Using actual densities of 1+ parr in December, the average number of parr available to migrate as smolts after 2 yr was 6.6 fish/100 m^2 in the North River and 0.8 fish/100 m^2 in the Westfield River (Table 3 and Fig. 6). Using the regression equations to estimate the number of parr on December 1, the average potential production of 2-yr smolts was estimated as 2.7 fish/100 m^2 in the North River and 0.8 fish/100 m^2 in the Westfield River (Table 2 and Fig. 6) The difference between both estimates in the North River was probably related to the influx of large parr in November increasing the density over that in previous months (Fig. 6). Parr that did not smoltify after 2 yr were very scarce in both rivers (mean annual densities of 2+ parr, 0.17 and 0.23 fish/100 m^2 in the North River and, 0.05 and 0.06 fish/100 m^2 in the Westfield River), and, therefore, did not greatly affect the estimates of potential smolt production. Regardless of seasonal

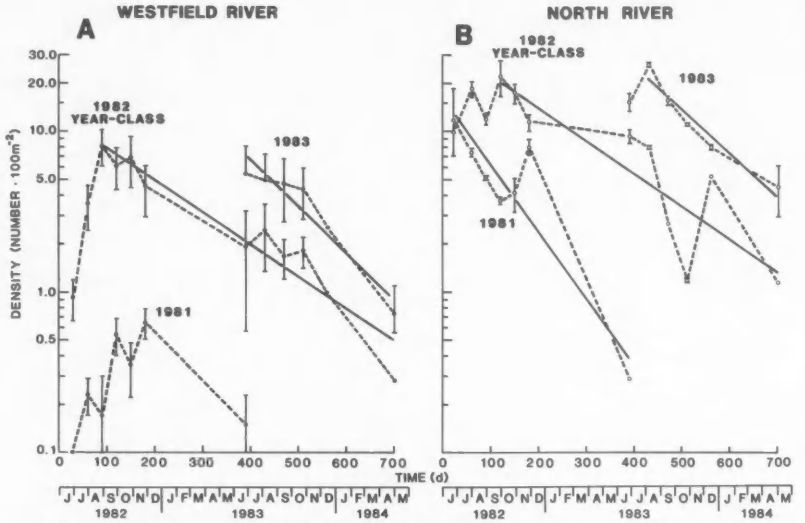

FIG. 6. Changes in the density of each year-class of juvenile salmon over time in (A) the Westfield River (mean density and SE from variance for each estimate and for mean) and (B) the North River (density and SE for each estimate). Solid lines are the least-squares regressions calculated from equations in Table 2 for the periods starting at times of maximum density.

TABLE 2. Regression statistics of $\ln N = a - Zt$, where N = fish density, Z = exponential rate of decrease, t = time in days from June 1, for year-classes during periods between maximum and minimum density (n = number of density estimates used in a regression). The density of fry (N_f, fish/100 m^2) on June 1 (1982 and 1983 year-classes) and the density of potential smolts (N_s, fish/100 m^2) on December 1 (1981 and 1982 year-classes) are estimated from the regressions.

	Year-class	n	$Z \pm$ SE	a	r	N_f	N_s
Westfield	1981	2	0.0068	0.7266			0.59
River	1982	10	0.0046 ± 0.0005	2.5213	−0.961**	12.44	0.99
	1983	6	0.0066 ± 0.0010	2.0996	−0.956*	8.16	
North	1981	7	0.0095 ± 0.0017	2.7479	−0.929*		2.72
River	1982	9	0.0047 ± 0.0010	3.5664	−0.872*	35.39	2.67
	1983	5	0.0062 ± 0.0010	3.4337	−0.965*	30.99	

*Correlation significant at $P<0.005$ (Student's t).
**$P<0.001$.

differences, mean annual densities of juvenile salmon were similar from year to year, within each river, and they were considerably lower in the Westfield River than in the North River (Table 4).

Some of the monthly variance in estimated density from the least-squares regression may be explained as the result of fish movements rather than mortality. The proportion of marked 0+ fish recovered was very low in July and increased to maximum at low water flows in October when the majority of fish could be considered as resident (Fig. 7). Dispersal of 0+ fish from areas of higher redd densities and a net immigration during the preceding months would account for some of the summer increase in density of 0+ fish in 1982 in both rivers and in 1983 in the North River, but part of this increase would probably be the result of a greater efficiency of capture as 0+ parr become larger and at low flows. A slight net immigration of 1+ parr to study sites in the Westfield River in the summer of 1982, possibly from downstream areas such as the less acidic Medway River, would explain the gradual increase in density of the 1981 year-class in the Westfield River (Fig. 6, 7). The increase, however, is minimal and densities were always extremely low (<1 fish/100 m^2). Net immigration of 1+ parr also decreased as water flows approached minimum levels in both rivers (Fig. 2, 7). There was a marked decrease in the proportion of resident 1+ parr in November–December as shown by the low recovery of marked fish in both rivers, suggesting that some emigration of these fish occurred. A change in microhabitat in autumn as water temperature decreased below 10°C (Fig. 4) could have resulted in a decrease in capture efficiency of resident parr. However, the increase in density of 1+ parr at that time was especially large in North River, indicating that capture efficiency was not decreased and that there was, instead, an influx of 1+ parr along with the emigration of resident parr. This situation would result in proportionally few recaptures. The influx of parr in late autumn in the North River consisted primarily of parr, large enough to be smolts the following spring, which probably came from areas upstream of the site. These parr would tend to congregate in

TABLE 3. Exponential rate of decrease (Z = mortality + net emigration, $\%\cdot d^{-1}$) of 0+ and 1+ salmon in the Westfield and North rivers for the summer–autumn period (between maximum summer density and that in November–December) and the winter–spring period (between November–December and May–June). Density (fish/100 m^2) at the beginning of each interval is in parentheses.

		0+		1+	
	Year	Summer–Autumn	Winter–Spring	Summer–Autumn	Winter–Spring
Westfield River	1982	0.69 (8.07)	0.40 (4.51)	— (0.64)	0.68 (0.64)*
	1983	0.46 (5.37)	0.84 (2.49)	0.72 (2.42)	0.86 (0.98)*
North River	1982	1.19 (21.97)	0.10 (11.42)	0.30 (12.81)	1.54 (8.03)*
	1983	0.95 (25.80)	0.40 (7.99)	0.35 (9.30)	1.04 (5.23)*

*Potential production of 2-yr smolts (fish/100 m^2).

the lower reaches of the river, including the study site 0.5 km from the mouth.

Movements of parr at the weir on the Westfield River indicate that in both years the main migration was downstream in the spring (Fig. 8), and these were mostly large parr and smolts. There were some movements of parr in both directions in the summer in 1982, but no movements after June in 1983 when summer flows were at their lowest during the study (Fig. 2). There were some movements of parr, mostly precocious males, in both directions during the spawning period in November, 1982, but not in 1983 (Fig. 8). There was no fall emigration of large parr destined to become smolts the following spring, at least before the weir was removed at the beginning of December when water flow increased drastically.

Growth

Changes in mean length and mean weight of 0+ and 1+ salmon from emergence to 2-yr parr indicate significant differences in the growth of fish between rivers (Fig. 9). Emergence of fry in the Westfield River was observed, May 14–25 in 1982, and mean length and weight with SD at emergence were 25.2 ± 1.3 mm and 0.14 ± 0.03 g, respectively. Fry were also seen on and near redds in the North River during the last week of May. In both years, 0+ salmon were initially larger in the North River during the last week of May. In both years, 0+ salmon were initially larger in the North River than the Westfield River, but by September–October their size was similar. Thereafter, 0+ fish grew faster in the Westfield River, and they were always slightly larger than those in the North River although the differences were not significant overwinter (Fig. 9).

Spring growth of 1+ salmon (March–June) was considerably greater in the Westfield River than in the North River, establishing a persistent size difference for the remainder of freshwater growth (Fig. 9). This is reflected by the differences in length and weight attained by 1+ salmon at the end of the growing season (Table 4). In both years, 1+ and 2+ parr were larger at the much lower densities in the Westfield River than those in the North River. Changes in length and weight of 1+ fish in both rivers reveal periods of rapid growth in spring and to a lesser extent in autumn, and little change in size or even apparent weight loss in July and August. Decreases in length and weight of 1+ salmon from December to February were possibly due to emigration of larger, faster growing individuals after 2 yr as a prelude to smoltification. The mean size of the remaining fish would then be biased towards smaller individuals. Differences in size attained from year to year in a river were considerable, especially for parr in the Westfield River, and were apparently unrelated to density (Table 4). The size of 0+ salmon was greater in 1983 than 1982 in both rivers, but the sizes of 1+ and 2+ fish were greater in 1982. A more extensive period of high water temperature and low water flow in 1983 than 1982 may have been responsible for these year-to-year differences in growth.

Daily instantaneous growth rates of each age-class show the variation in the rate of change in body weight in relation to age

TABLE 4. Mean lengths and weights of juvenile salmon with SD in parentheses and sample size (N) at the end of the 1982 and 1983 growing seasons (i.e., in November–December) and mean densities (fish/100 m^2) by age-class over the growth periods in the Westfield and North rivers.

		Westfield River				North River			
Age-class	Year	Density	Length (mm)	Weight (g)	N	Density	Length (mm)	Weight (g)	N
0+	1982	4.98	63.3 (5.8)	2.61 (0.79)	286	15.09	63.2 (4.9)	2.76 (0.74)	57
	1983	4.37	70.7** (5.3)	3.79** (0.79)	9	15.15	65.8** (4.4)	2.75* (0.58)	38
1+	1982	0.33	135.0** (12.2)	26.9** (6.9)	43	6.85	114.8** (13.3)	16.9** (6.0)	40
	1983	1.75	123.1** (11.0)	18.5* (4.3)	15	5.25	112.0** (13.6)	15.5* (4.9)	28
2+	1982	0.05	174.6 (6.8)	57.9 (7.9)	12	0.23	155.5 (3.5)	40.2 (1.0)	2
	1983	0.06	149.0 (1.7)	35.4 (2.2)	3	0.17	138.0 (0)	27.7 (1.3)	2

*Significant differences in mean length and mean weight of 0+ and 1+ fish between rivers (t-test, $P<0.05$).
**$P<0.01$.

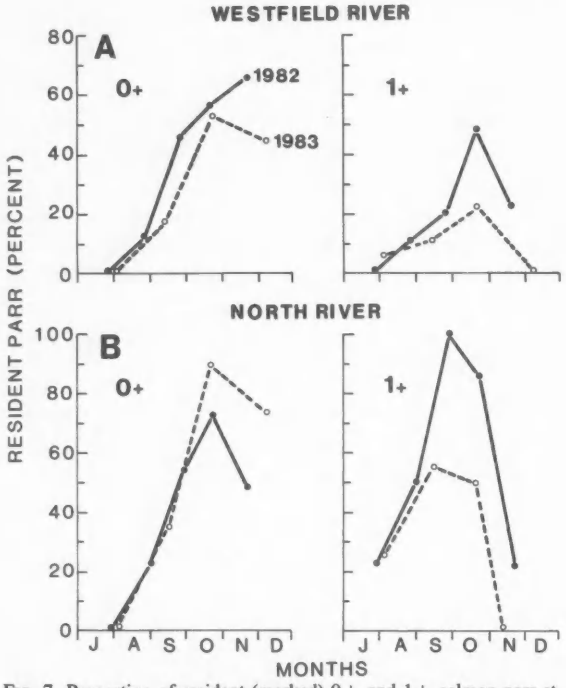

FIG. 7. Proportion of resident (marked) 0+ and 1+ salmon parr at times of density estimates during 1982 (solid lines) and 1983 (broken lines) in (A) the Westfield River and (B) the North River.

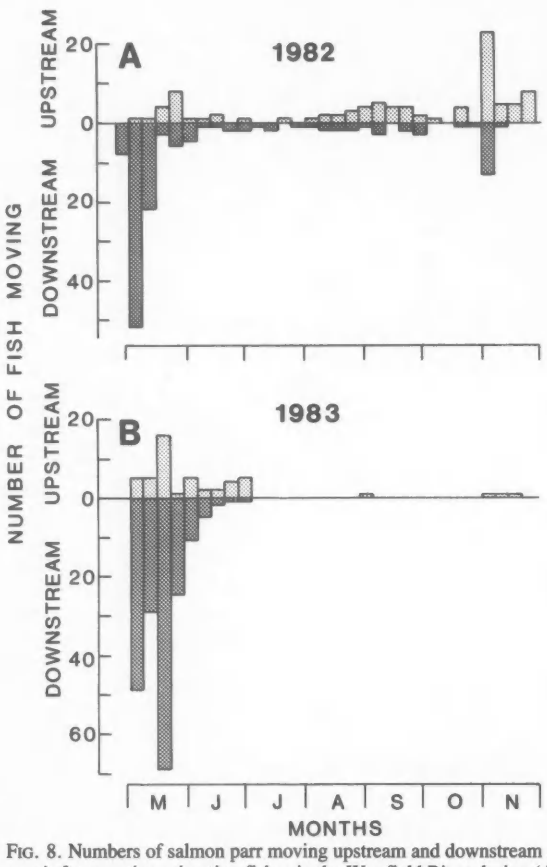

FIG. 8. Numbers of salmon parr moving upstream and downstream at a weir for counting migrating fishes in the Westfield River during (A) 1982 and (B) 1983. Daily numbers are combined into weekly frequencies.

and season for both rivers (Fig. 10). The highest growth rates were measured after emergence in June–July for 0+ salmon and in April–June for 1+ fish. There were differences in timing between years of sampling, but the trends were similar. Growth rates for 0+ fish continually decreased until winter minimum values were reached. In contrast, growth rates of 1+ groups decreased to low and slightly negative values during July and August, but increased to a second smaller peak from September to November and decreased again in December, indicating dual growth phases (Fig. 10). Samples of 2+ parr were too small or infrequent to yield reliable period-specific growth rates.

Growth rates calculated for the intervals shown in Fig. 9 were related to water temperature during these intervals and the relationships were different for each age-class (Fig. 11). Because a detailed daily temperature record was only available for the Westfield River, the relationships were not examined for the North River. Growth rate of 0+ parr increased with mean temperature (Fig. 11A; $G_w = -0.152 + 0.100T, r = 0.812, P < 0.001$), and 66% of the variation in growth was attributable to water temperature. However, maximum growth rate was probably as much a function of rapid early development coinciding with periods of relatively high temperature and long day-length. Growth rates of 1+ fish increased only between 8–18°C and were usually near zero or less at temperatures beyond this range (Fig. 11B; $G_w = -0.064 - 0.109T + 0.032T^2 - 0.0017^3, r = 0.626, P < 0.01$). This points to the adverse effect of high temperatures on the growth of 1+ parr and lends some support to the premise of temperature-related biphasic growth.

Production

Fry emergence, considered to be complete by June 1 each year in both streams, was taken as the initial point in the calculation of production for the 0+ age-class. Calculations were based on densities at that date, extrapolated from the regression of density on time (Table 2), and the mean weight of fry at emergence in the Westfield River (0.14 g). Production of an age-class was assumed to extend to the end of the growth period (November–December), and production during the winter–spring interval (November–December to May–June) was attributed to the next age-class because most of the production was considered to occur in spring during the period of rapid growth (Fig. 9, 10).

The production of each year-class in both rivers was calculated for each interval between density estimates (Table 5). Mean production in the Westfield River was obtained by averaging the production values for each site, calculated separately using the actual biomass at each site and the growth rates calculated for all sites combined because sample size in each site was often too small to yield reliable interval growth rates. The range of production values for sites in the Westfield River indicates that production at some sites was extremely low and that maximum production was almost always less than the production in the North River. Production varied greatly among intervals in each river, but it was highest in both rivers during the winter–spring interval because of the very high growth rate of 1+ parr during May–June. During the summer months, 0+

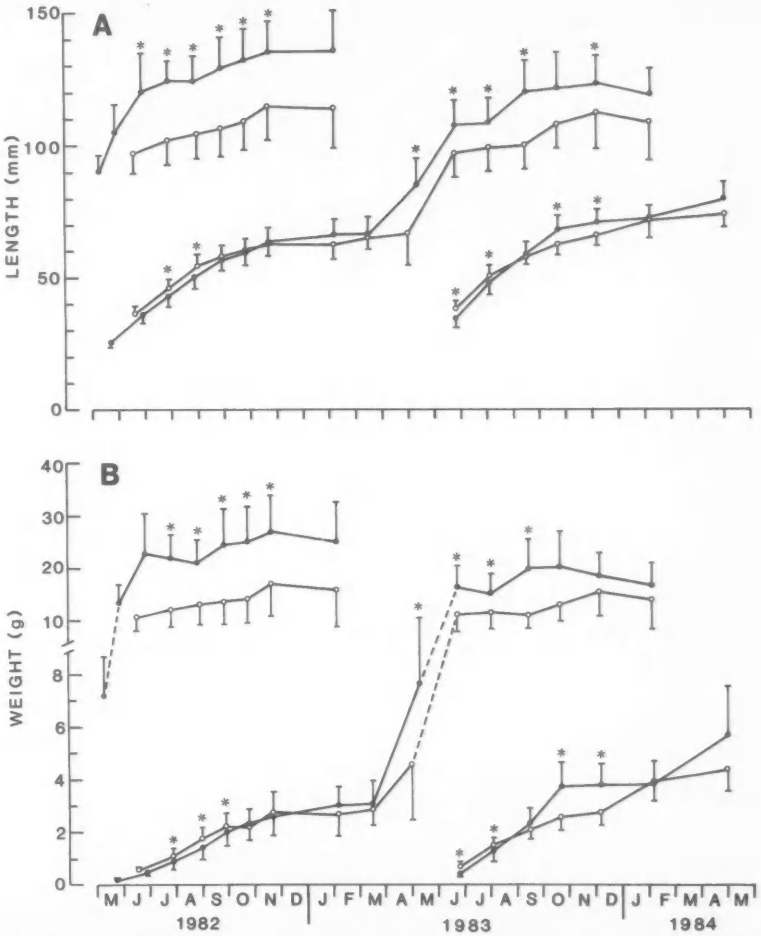

FIG. 9. Growth of each year-class of juvenile salmon expressed as changes in (A) mean length (+ or − SD) and (B) mean weight (+ or − SD) for combined samples in the Westfield River (●) and in the North River (○). Asterisks indicate samples with significant differences between rivers (*t*-test, $P < 0.01$).

parr accounted for most of the production because of their continued growth throughout the summer compared with the poor growth and even apparent loss of weight by 1+ parr over the same period (Fig. 9, 10).

Production by an age-class was generally similar from year to year during comparable intervals (i.e., similar time and duration). However, the apparently lower spring production of 1+ parr in 1984 (i.e., 1983 year-class) than in 1983 (i.e., 1982 year-class) was a result of differences in duration of the interval. In 1983, it extended to the end of June, whereas in 1984, it only extended to early May, thereby missing the major growth increment.

The total annual production of salmon averaged 135 g·100 m^{-2} in the North River over the two-year period and only about 40 g·100 m^{-2} in the Westfield River (Table 6). The combined production of 0+ and 1+ parr comprised the greater amount of the total production (about 90% or more). Each age-class contributed similar proportions to the total annual production in both rivers. The proportion of the total annual production provided by 1+ parr (>55%) was, in both rivers, close to twice that contributed by 0+ parr. This dominance of the annual production by 1+ parr is considered to be mostly a function of the very large growth increments of this age-class. Annual production of 2+ parr was slight despite their large size because of the very low densities because of the dominance of 2-yr smolts in both rivers.

The yield of 1+ parr at the end of the growth period of the second year, which is used here as an indication of maximum smolt production, was 5 fish/100 m^2 in the North River and <1 fish/100 m^2 in the Westfield River, for the 1982 year-class (Table 7). Total production of the 1982 year-class from emergence to 2+ parr was almost three times higher in the North River than it was in the Westfield River. A comparison of this total production of salmon of that year-class with the yield of presmolts indicates that almost 60% of all production in the North River was in the form of presmolts compared with about half of that value in the Westfield River (Table 7).

The biomass of salmon at the end of the growing season of each year was higher for all age-classes in the North River than in the Westfield River (Table 8). This accounts for the similar differences in production rates between rivers, given that the growth rates of parr were either similar (0+ age-class) or con-

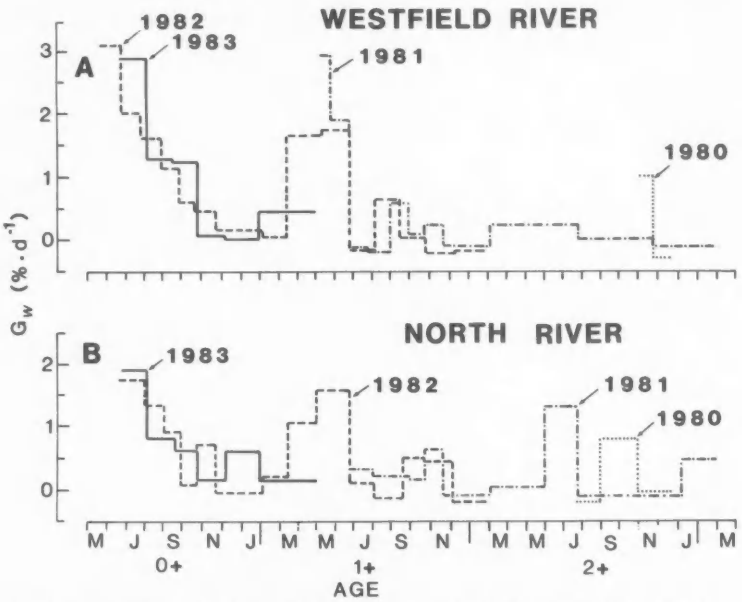

FIG. 10. Seasonal variations in the daily instantaneous growth rates (G_w, weight) of 0+, 1+, and 2+ salmon parr in (A) the Westfield River and (B) the North River from data for the following year-classes: 1980 (·····); 1981(–·–·–); 1982 (- - - -); 1983 (____).

siderably greater (1+ age-class) in the Westfield River than in the North River. The biomass of 1+ parr was greater than that of 0+ salmon in both rivers. The total biomass of salmon was higher in both rivers in 1982 than it was in 1983, but the proportional differences between rivers were generally maintained from one year to the next.

Discussion

Factors governing the production of juvenile Atlantic salmon, i.e., those that affect either growth rate or population density, have generally been recognized as chemical, physical (temperature, depth and velocity, type of bed materials) and biotic (food resources, competition, recruitment) (Egglishaw and Shackley 1985). Several approaches may be used to determine which factor(s) are responsible for differences in survival, growth, and production of juvenile salmon between rivers. These could involve habitat and population manipulations, statistical analysis such as key factor analysis in a large sample of rivers where most factors vary more or less randomly among the rivers, or experimentation with selected critical factors to examine their effects on the different life-history stages from egg deposition to smolt. The latter approach was used *in situ* to examine the effects of water chemistry, specifically those associated with pH changes, on juvenile salmon in acidic rivers of Nova Scotia (Lacroix 1985; Lacroix et al. 1985; Lacroix and Townsend 1987; Lacroix et al. 1990). Fish community structure (e.g., numbers and types of competing species) was also examined in relation to water chemistry differences in these rivers (Lacroix 1987b). In the present study, except for pH and associated factors, differences in physical and biotic factors among study sites and between rivers were kept to a minimum where possible and within the range of habitat variables selected by juvenile salmon for those factors (e.g., bed material size, depth, and velocity) for which a selection has been shown (Egglishaw and Shackley 1982; Kennedy and Strange 1982; Rimmer et al. 1984; Morantz et al. 1987). The effects of temperature on growth rate and their implication for a comparison of production between rivers is discussed as are the effects of density (i.e., intra-specific competition) on the survival and growth of fish. A companion study of the food resources available and consumed by juvenile salmon in the Westfield and North rivers was also conducted during 1982–84, and it will be separately published; briefly, although there were differences in the species composition of the benthos and drift, the overall biomass of food items available to juvenile salmon did not differ between rivers and food consumption was apparently not a factor limiting production (G. L. Lacroix, unpublished data). The presence of predatory birds and of other predators was rarely noted in more than seven years of intensive work on the rivers (G. L. Lacroix, personal observations), and their importance as factors capable of accounting for differences in production between rivers is considered to be negligible. Density, growth, and production in feral populations of juvenile salmon in acidic rivers are, therefore, compared on the basis of the findings from previous *in situ* experiments, the lack of major physical differences other than pH, and the driving effects of pH on the biotic factors of importance.

Production of juvenile Atlantic salmon was considerably lower at mean pH 4.9 in the Westfield River (<0.4 g·m^{-2}·yr^{-1}) than at mean pH 5.9 in the North River (1.4 g·m^{-2}·yr^{-1}), and maximum production rates attained in sites of the Westfield River were always less than rates at higher pH levels in the North River. These low production rates in the more acidic Westfield River were attributable to the effect of very low densities in that river rather than growth because growth was actually faster in the more acidic of the two rivers. Production at pH levels of 5.6–6.3 in the North River was com-

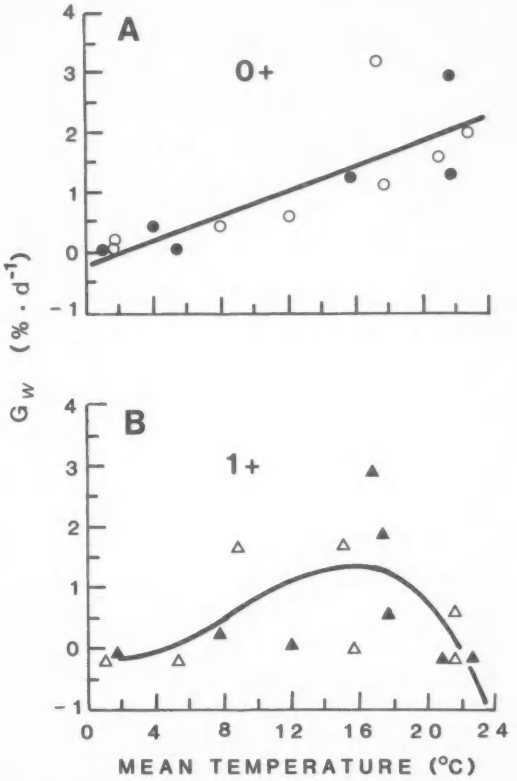

FIG. 11. Variations in the daily instantaneous growth rates (G_w, weight) of (A) 0+ and (B) 1+ parr as a function of mean water temperature during the period for which growth rate was calculated in the Westfield River. Curves are fitted for the regressions calculated. Data are from the following year-classes: 1981 (△); 1982 (●,▲); 1983 (○).

parable to that in some areas with alkaline waters or near-neutral pH levels in Canada (Randall and Paim 1982; Randall and Chadwick 1986) and the U. K. (Gee et al. 1978b). Annual salmon production in the North River was also intermediate to that in two West Norwegian rivers with mean pH levels of 5.5 and 5.7 (Fjellheim et al. 1987). Production at pH levels of 4.7–5.4 in the Westfield River was at the lower end of the range reported for self-sustaining Atlantic salmon populations in North America and Europe (summarized in Randall and Paim 1982) and for those at the northerly limits of distribution (Power 1973).

Almost 60% of the total production by a year-class potentially contributed to smolt production in the North River, but only 35% of it did in the more acidic Westfield River. The dominant smolt age in these systems was 2 yr (average 91%). This is further supported by Wykes' (1967) report that 89.5% of smolts in the Medway River and its tributaries were 2 yr old or less. Potential production of 2-yr smolts averaged 0.8/100 m^2 in the Westfield River, but the average was between 2.7–6.6/100 m^2 in the North River, depending upon the method of estimation. Actual smolt production in the North River would be slightly lower if overwinter mortality was taken into account. It was then probably similar to averages summarized by Baglinière and Champigneulle (1986) for smolts of similar age in rivers of North America (3.7 smolts/100 m^2) and Great Britain (4.6 smolts/100 m^2). However, an actual smolt production of <0.8/100 m^2 in the more acidic river would, in the absence of strays of hatchery origin in the escapement (average 65%), be insufficient for the continued maintenance of a salmon population in that river. This is based on the stock/recruitment relationships and the minimum replacement line described for acidic Nova Scotia rivers by Lacroix (1987a).

Production in both the Westfield and North rivers was greater in the second year (1+), 56 and 59%, respectively, than it was in the first (0+), 38 and 32%, respectively. Production during the first year occurred over the summer months, whereas much of the second year's production occurred during the spring interval. In contrast, Egglishaw and Shackley (1977) determined production of salmon in a stream in Scotland to be generally greater in the first than in the second year of life, and Prouzet and Gaignon (1982) reported that 72% of salmon production in a river of Northern Brittany was also in the first of two years, with maximum production in spring and minimum in winter. Backiel and LeCren (1978) indicated that the largest contribution to production in salmonids is generally from the youngest age-group because density rather than growth has the greatest influence on production and that variation in growth, whether density-dependent or not, seldom has a significant effect on production. Production should thus increase with increased population density and vice-versa, and compensatory faster growth in low density populations, e.g., in the Westfield River, should not have a very significant effect on increasing overall production. Nevertheless, the higher contribution to production by 1+ parr in both rivers, in contrast to the other studies, could in part be related to a more extensive growth period during the second year.

Growth was not limiting to production in the Nova Scotia rivers, especially at the lowest pH levels where growth was greatest. Size attained by parr of all age-classes at the end of the growing season was considerably greater than in northern New Brunswick rivers (Randall and Paim 1982; Randall and Chadwick 1986) and comparable to some of the largest sizes reported in Europe and the U.K. (Egglishaw and Shackley 1977; Gee et al. 1978b; Baglinière 1979; Prouzet and Gaignon 1982). Lower limits to production were, therefore, the result of lower densities. Mean densities of juvenile salmon were very low in the Westfield River in relation to those in the North River. The density of 0+ parr, one month after emergence, was on average about 70% lower in the more acidic river. Fry density is usually considered to be limited by number of spawners (Gee et al. 1978a). However, fry densities in the Westfield River were low regardless of the relatively high redd density and egg deposition in that river compared to those in the North River. A higher overall egg deposition per unit area in the Westfield River than in the North River has been consistently observed between 1981 and 1986 (Lacroix 1987a). Because of low smolt production, this higher egg deposition per unit area in the Westfield River is considered to be related mostly to the straying of salmon originating from enhancement in other areas in the Medway River or from more successful feral populations in less acidic downstream areas of that river. Considering the 53% average hatching success of eggs in the Westfield River (Lacroix 1985), the low fry densities can be attributed mainly to the pH-related high mortality of postemergent fry at pH 5.0 measured in spring on the Westfield River (Lacroix et al. 1985).

TABLE 5. Production (g·100 m^{-2}/interval) of juvenile salmon of each year-class in the Westfield River (mean and range for five sites in 1982 and three sites in 1983–84) and the North River during the 1982–84 study period. Production of the 0+ age-class was calculated from June 1 for the 1982 and 1983 year-classes.

Year and interval of production	Year-class				Interval total
	1980	1981	1982	1983	
Westfield River					
1982 June[c]	—	—	1.07 (0.64–1.70)	—	1.07
July[c]	0	0	0.80 (0.26–1.27)	—	0.80
Aug.[c]	0	0	2.56 (0–4.43)	—	2.56
Sept.[c]	0	0.94 (0–1.95)	3.46 (0.77–6.44)	—	4.40
Oct.[c]	0.29 (0–1.47)	0.21 (0.03–0.36)	2.08 (0.58–4.05)	—	2.58
Nov.[c]	0	0.79 (0–1.52)	1.80 (0.50–3.69)	—	2.59
1983 June[c]	0	2.42 (0.90–3.18)	30.76 (8.62–48.4)	2.10 (1.78–2.41)	35.28
Aug.[a]	—	0	0	4.84 (0–7.63)	4.84
Sept.[b]	—	0	9.25 (5.65–14.9)	4.59 (0–7.18)	13.84
Oct.[c]	—	0	0.51 (0.37–0.75)	6.31 (0.99–9.14)	6.82
Dec.[a]	—	0	0	0.14	0.14
1984 May[a]	—	0	0	3.88 (1.70–5.86)	3.88
North River					
1982 June[c]	—	—	6.89	—	6.89
July[c]	0	14.69	8.02	—	22.71
Aug.[c]	0	4.91	9.23	—	14.14
Sept.[c]	0	3.03	7.66	—	10.69
Oct.[c]	0	1.95	0.78	—	2.73
Nov.[c]	0	16.55	7.29	—	23.84
1983 June[c]	—	32.87	84.12	11.30	128.29
Aug.[a]	—	0	3.43	16.56	19.99
Sept.[b]	—	0	0	11.63	11.63
Oct.[c]	—	0	3.42	6.58	10.00
Dec.[a]	—	0	7.82	1.60	9.42
1984 May[a]	—	0	0	9.87	9.87

[a]Interval ending at the start of the month.
[b]Interval ending at the middle of the month.
[c]Interval ending at the end of the month.

TABLE 6. Average annual production (g·100 m^{-2}·yr^{-1}) of juvenile salmon and percentage contribution made by each age-class to the total annual production in the Westfield River (mean for sites) and the North River for the period between June 1982 and May 1984.

Age-class	Westfield River	North River
0+	14.9 (38%)	43.8 (32%)
1+	23.2 (59%)	74.9 (56%)
2+	1.4 (3%)	16.4 (12%)
Total	39.5	135.1

Table 7. Yield (fish/100 m^2 and g·100 m^{-2}) of 1+ juvenile salmon at the end of the growth period of the second year and total production from emergence to presmolt for the 1982 year-class in the Westfield River (mean for sites) and the North river.

	Yield		Total production of 1982 year-class	% of year-class production as presmolts
	Number	Weight		
Westfield River	0.98	18.1	52.3	34.6
North River	5.23	81.1	138.7	58.5

After the initial loss of postemergent fry, overall rates of loss over the remainder of the freshwater phase appeared similar in both rivers, but a closer examination of seasonal changes indicated differences in mortality rate between streams during specific intervals. Different rates or phases of salmon loss can occur during the juvenile stages. Egglishaw and Shackley (1977) observed three to four successive stages where mortality rates of juvenile salmon change abruptly and are significantly different. The summer loss of 0+ parr was higher at the high densities in the North River than in the more acidic Westfield River. Density-dependent factors possibly acted to reduce the

TABLE 8. Biomass ($g \cdot 100\ m^{-2}$) of juvenile salmon at the end of the 1982 and 1983 growing seasons (i.e., in November–December) in the Westfield and North rivers and percentage by age-class in parentheses.

		Age-class			
	Year	0+	1+	2+	Total
Westfield River	1982	11.77 (29)	17.22 (43)	11.00 (28)	39.99
	1983	9.44 (29)	18.13 (55)	5.31 (16)	32.88
North River	1982	31.52 (17)	135.71 (71)	22.51 (12)	189.74
	1983	21.97 (18)	81.06 (68)	16.07 (14)	119.10

density of 0+ fish (through immigration and death) at the high densities of 0+ and 1+ parr in the North River, but apparently not at the overall low densities of parr in the Westfield River. Summer is the period of highest pH in that river and additional pH-related mortality was not expected. The overwinter mortality rate of 0+ parr was more than twice as high in the Westfield River than it was in the North River. The pH levels in the Westfield River were < 5.0 from December to April and at times as low as pH 4.7 and approaching the critical level at which parr in rivers of Nova Scotia will die because of ionoregulatory failure. Any pH decrease below 4.7 will severely reduce densities and completely eliminate year-classes of both small and large parr if the duration exceeds about 20 d at pH 4.6–4.7 or several days at pH 4.4–4.6 (Lacroix and Townsend 1987). Therefore, pH levels approaching these low lethal levels are possibly responsible for higher mortality in the Westfield River. As a result, early summer densities of 1+ parr were ≤2 fish/100m^2 in the Westfield River compared to ≥10 fish/100 m^2 in the less acidic river. Overwinter loss of 1+ parr was either equal to or higher than losses at other times in both rivers, and it was almost twice as large in the North River as in the Westfield River. This high loss is probably related more to the high net emigration of parr ready to smoltify after 2 yr than to high mortality during that period based on the high proportion of 2-yr smolts in these rivers. Because proportionally fewer fish are left after 2 yr to emigrate as smolts in the Westfield River than in the North River, the loss was apparently lower in the former. Egglishaw and Shackley (1977) also observed an increase in the rate of salmon loss after November of the second year as presmolts emigrate.

Density of parr in the North River was only slightly less than the optimal value for 1+ parr (13/100 m^2) taken from Elson's (1975) model for adequately stocked habitat, even though initial densities of fry were lower in the North River than those in Elson's model (69/100 m^2), or the density of fry (75/100 m^2) considered by Gee et al. (1978a) to be optimal for maximum production of presmolts. Although it would have been preferable if more than one site was sampled in the North River such as in the Westfield River, it was, nevertheless, representative of other sites (both adjacent and further upstream) sampled in previous years in that river. Summer densities of wild parr (all ages) in the North River were 11.9–24.8/100 m^2 in 1976 and 1977 (Gray et al. 1978), 27.1–27.7/100 m^2 in 1978 (Cameron and Gray 1979), and 26.0–33.7/100m^2 in this study. In contrast, densities of juvenile salmon at low pH in the Westfield River were very low, mostly as a result of the low initial densities of 0+ parr after the high mortality of postemergent fry at pH 5.0 (Lacroix et al. 1985).

In both Nova Scotia rivers, the exponential decrease in abundance of year-classes of salmon with time was preceded by a short recruitment period in June. This is similar to the pattern observed in New Brunswick rivers (Randall and Paim 1982). The increase in 0+ parr density in the early part of summer in both rivers, most years, probably resulted partly from net immigration because of dispersal of fry from areas of high redd density and partly from an increase in electrofishing efficiency as fry grew and as water levels fell. The decrease that followed, however, probably resulted mostly from mortality by factors that normally regulate density (e.g., disease, predation, starvation, or physical factors) and by acid stress in the Westfield River. Although there may be an initial period of emigration and dispersal from redd areas, the indication from marking and recapture data in this study and from the weir for counting upstream and downstream migrants in the Westfield River is that during the summer there was an increasing number of residents and few movements, and that little emigration of parr occurred before the winter–spring (i.e., preceding and during smoltification). Randall and Paim (1982) recorded similar movement patterns in the New Brunswick sites where they captured a large proportion of immigrants (especially 2-yr-old fish) in fall samples. This is similar to the situation in the Nova Scotia rivers. Parr movements are generally restricted at low flows and in summer (Saunders and Gee 1964; Egglishaw and Shackley 1977), a condition that prevails from July to October in the two study streams, whereas downstream migrations of salmon parr can occur in autumn at high flows (Meister 1962; Mills 1964; Saunders 1976; Buck and Youngson 1982). The rates of salmon loss from streams are generally considered to be close to the instantaneous natural mortality rates during the first 2 yr of life after which emigration, as a prelude to smoltification, becomes a more likely cause of loss (Egglishaw and Shackley 1977; Gee et al. 1978a). Movements of parr to areas more favorable to growth (Egglishaw and Shackley 1980) were unlikely considering the fast growth, especially in the more acidic river. Therefore, increased mortality of fry in acid water, rather than emigration, was considered as the main cause of the weak older year-class. The numbers of fish in older year-classes are usually a function of the year-class strength of original fry (Gee et al. 1978a). In the North River, however, the high first summer disappearance rates compared with those in the Westfield River after the loss of postemergent fry, may also be related to the fact that at higher densities in the North River, disappearance rates (mortality + emigration) can be positively density-dependent (Egglishaw and Shackley 1977; Gee et al. 1978a; Randall and Chadwick 1986). When resources are limiting, mortality, growth, and production of salmonids can be regulated by density-dependent factors (LeCren 1973; Mortensen 1977; Backiel and LeCren 1978; Elliott 1984, 1985; Randall and Chadwick 1986).

Biomass of juvenile salmon of all age-classes was less (by more than 70%) in the Westfield River than it was in the North River, despite the larger size of parr in the former, because of the large differences in density. Although the size of salmon fry was initially, larger in the North River, parr were progressively larger in the Westfield River after 2–3 mo. The size attained by 1+ parr was 10–20% greater in the Westfield River than in the North River. This eventually resulted in very large 2-yr smolts in the Westfield River (mean length measured at the weir, 18.4 and 18.2 cm in 1982 and 1983, respectively). The smaller initial size of 0+ parr in the Westfield River compared to those in the North River may be due to the adverse effect of low pH on yolk utilization yielding smaller fry (Peterson and Martin-Robichaud 1986), or to the relatively larger proportion of 1-sea-winter (1SW) spawners or small fish in the

escapement in the Westfield River. Egg size is generally positively correlated with fish length for Atlantic salmon (Pope et al. 1961) such that the progeny of 1SW spawners would be relatively smaller at emergence than that of larger multi-sea-winter (MSW) salmon. The average 1SW:MSW ratio was 9:1 in the Westfield River and it was about 6:1 in the LaHave River (at Morgan Falls, above which the North River enters). After the first winter, 1+ parr were much larger at the lower pH levels, the faster growth possibly resulting from the low density of parr. Egglishaw and Shackley (1977;1980) determined that the growth and mean weight of salmon at the end of the first growing season were inversely related to both their population density and the biomass of 1+ salmon present. Furthermore, the mean weight of 1+ salmon was highest in sections where 0+ salmon had reached a relatively large size the previous year. This would be similar to the situation in the Nova Scotia rivers where growth was faster and sizes at the end of the season were larger in the more acidic stream at low density of 0+ and low biomass of 1+ salmon. In contrast to this study. Fjellheim et al. (1987) observed no relationship between density and growth and no growth difference between salmon parr, regardless of age, in two acidic rivers in spite of episodes of greater acidity in one of the rivers.

The effects of acidity on the growth of fish in freshwater can be varied; growth can either be increased, decreased, or unaltered, probably through the combined effects of metabolic stress, fish popultion density, and food supply (reviewed by Harvey 1982). For a majority of fish species in acidified waters, the growth of surviving fish, following a mortality period or as a consequence of reduced reproductive success, may be improved as a result of reduced competition for food in sparse populations if food supplies are not impoverished by acidification (Dannevig 1966; Almer 1972; Jensen and Snekvik 1972; Ryan and Harvey 1977; Almer et al. 1978; Andersson and Andersson 1984). Sadler and Lynam (1987) indicated that low pH alone has relatively little effect on brown trout (*Salmo trutta*) growth in laboratory bioassays until lethal levels are reached (pH 4.3), and only then does growth rate decrease. The faster growth of parr in the Westfield River than in the North River is not to say that growth would not be impaired when the pH approaches lethal levels as shown by a drastic reduction in feeding by fry and parr under such conditions (Lacroix et al. 1985; Lacroix and Townsend 1987).

The possibility of size-selective mortality (Ricker 1969) or size-dependent movement (Egglishaw 1967; Chapman 1978a) affecting growth rate estimates especially in acid streams where selective death of small fish would bias growth estimates to show apparent large mean size and faster growth should not be overlooked. The faster growth in the Westfield River may partly result from a high mortality rate of the smallest fry, the survival of large healthier fry creating a bias towards an apparently faster growth of the population. Small parr have a shorter resistance time at low pH than larger parr (Lacroix and Townsend 1987), and are therefore considered to be more sensitive. Furthermore, salmon densities in both rivers were relatively low enough that they may have been below the critical densities where one might expect to see density-dependent responses. Elliott (1984, 1985) could not find any clear evidence of density-dependent growth in his study of brown trout (*Salmo trutta*) even with highly variable and very high densities. Thus, the hypothesis of size-selective mortality may also be a likely explanation for the larger size of parr in the more acidic river.

In addition to the effects of density, Egglishaw and Shackley (1985) also considered the effects of temperature on growth rate to be important. Temperature is generally considered important as a controlling factor for the growth of salmonids both in captivity (Elliott 1975a, 1975b, 1982) and in streams (Edwards et al. 1979). The biphasic growth of 1+ parr was apparently temperature-related and most likely a result of the adverse effects of high temperature on food consumption and conversion efficiency. The growth rate of 1+ parr increased to maximum above 8°C, but decreased to zero or less above 18°C. Dwyer and Piper (1987) calculated that 13–16°C are optimal for maximum growth of juvenile Atlantic salmon. However, the growth rate of 0+ parr in this study did not decrease in summer at greater than 20°C, but it continued to increase with water temperature, indicating that the optimal temperature for growth was higher for 0+ than for 1+ parr. The seasonal rhythm of growth for 1+ parr was also observed by Prouzet and Gaignon (1982) for both 0+ and 1+ age-classes, the summer decrease in growth being more pronounced for 1+ parr. They indicated that the large summer growth decline during the second year corresponded to a period of low flow ($0.3\ m^3 \cdot s^{-1}$) and high temperature (21°C). The biphasic growth pattern in the Nova Scotia rivers resulted in about 5–6 mo of growth and was probably the cause of the much greater size of 1+ parr than that in northern New Brunswick rivers where most growth is restricted to a short period of 3 mo in summer (Randall and Paim 1982).

This ecological study adds further support to the interpretations and conclusions made from previous *in situ* toxicity tests for specific life-stages of Atlantic salmon (Lacroix 1985; Lacroix et al. 1985; Lacroix and Townsend 1987), and it confirms the nature of low pH effects in acidified rivers of Nova Scotia on the dynamics of salmon populations in freshwater. Because of the relationship between production and biomass, factors affecting density, such as acid stress, directly affect juvenile salmon production. They may cause production to be reduced and even fall beyond the lower limit for maintenance of the population. In the Westfield River this happened when pH levels declined to 4.7 and remained within the 4.7–5.4 range. It is also apparent that low pH will drastically limit reproductive success to the point that a stock will probably disappear before there are any serious adverse trophic effects. The majority of acidified rivers in Nova Scotia where salmon populations are adversely affected have brown, humic waters rich in aluminum, but organic forms of aluminum which are nontoxic are dominant at all times, even during episodes of increased acidity (Lacroix and Kan 1986). The main lethal factor directly affecting density and, as a result, production is considered to be hydrogen ion activity (Lacroix and Townsend 1987; Lacroix et al. 1990).

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REVIEW/SYNTHÈSE

The Monotypic Sardines, *Sardina* and *Sardinops*: Their Taxonomy, Distribution, Stock Structure, and Zoogeography

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This paper reviews the taxonomy, distribution, stock structure, biochemical genetics and zoogeography of the world's two subtropical sardines, *Sardina* and *Sardinops*. We conclude that both genera are monotypic and that the differences between populations of *Sardinops* in different current systems are not large enough to consider them distinct subspecies. Analysis of biochemical genetics information, fossil evidence, and present and paleoclimatic conditions suggests that *Sardinops* did not achieve its present distribution until the Pleistocene and that westward exchange of sardines via the northern and southern west wind drift regions is possible with present climatic conditions. Transequator exchange appears to be possible only during glacial maxima and only in the eastern tropical Pacific.

Notre étude fait le point sur la taxinomie, la répartition, la structure des stocks, la génétique au niveau biochimique et la zoogéographie des deux sardines subtropicales du monde, *Sardina* et *Sardinops*. Nous en concluons que les deux genres sont monotypiques et que les différences entre les populations de *Sardinops* dans les divers systèmes de courants ne justifient pas leur séparation en sous-espèces distinctes. L'analyse des données génétiques, des éléments fournis par les fossiles et des conditions présentes et paléo-climatiques semble indiquer que *Sardinops* n'a réalisé sa présente répartition qu'au pléistocène et que l'échange de sardines vers l'ouest via les régions nord et sud de dérive due aux vents d'ouest est possible dans les conditions climatiques actuelles. L'échange à l'équateur ne semble possible que pendant les maxima glaciaires et dans l'est du Pacifique tropical.

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A small assemblage of species dominates the subtropical fish faunas of the world's eastern boundary currents (Bakun and Parrish 1980). The assemblage includes four types of small pelagic fishes; sardine (*Sardina* and *Sardinops*), anchovy (*Engraulis*), mackerel (*Scomber*), and jack mackerel (*Trachurus*). The same assemblage also dominates the epipelagic fish faunas of the subtropical portions of most of the western boundary currents. The most significant exception to this is the Gulf Stream region where sardines (*Sardina* or *Sardinops*) are absent and *Engraulis*, while abundant, is not a dominant species. The Brazil Current–Falkland (Malvinas) Current region also lacks *Sardina* or *Sardinops*, but the other types dominate the subtropical, epipelagic fish fauna in this region.

The four types account for about 30% of the world's catch of marine fishes. For most of the last 25 years either *Sardinops* or *Engraulis* has supported the world's largest fisheries and *Sardinops* is presently the largest single component of the world's harvest of fishes.

There has been a long history of economic and scientific interaction between the major fisheries on this assemblage of fishes, both within geographical areas and among areas. Fishing vessels catching one of these fishes usually also catch the others. The world's trade in fish meal is dominated by these fishes and they comprise a large proportion of the world's canned fish. Information, technology and material used in the fisheries for these fishes has been transferred from one area of the world to another for many decades. For example, the development of the California pelagic fishery was heavily influenced by canning technology developed for sardines in Europe (Thompson 1921) and the round-haul nets used in the early

California fishery were originally developed in Rhode Island, Italy, and Japan (Higgins and Holmes 1921; Scofield 1924). Much of the modern purse seine technology (Fry 1931), large-scale fish meal plants (Scofield 1921), and the first factory reduction ships (Scofield 1938) were developed in California. When the California sardine population collapsed much of the technology, and in many cases canning and fish meal machinery were transferred to South Africa, Peru, and Chile which fostered further development of both harvesting and processing technology. During the recent development of the Chilean sardine and jack mackerel fishery there were significant technological and investment transfers from South Africa. In Europe and Japan, where there were longer traditions of marine fisheries and local markets, development has had less interaction with other regions.

Transfer of research methodology and results between the research establishments in the various regions has also been extensive and continuous. However, whereas the transfer of methodology has been acccomplished very rapidly it is unclear whether the results from research in one area can be transferred to another area. One of the reasons for this is that there is an understandable tendency to assume that widely dispersed populations of admittedly very similar fishes (for example the various *Sardinops* populations) might be expected to have evolved adaptations which would be specific to their individual habitats. This assumption has been reinforced by the continued use of different species names in different regions.

With few exceptions, early taxonomists described a different species of sardine, mackerel, anchovy, and jack mackerel for each current system; in many cases they were originally described as different genera. Within each of the four groups of fishes the recent trend of taxonomy has been towards a reduction in the number of species. As will be shown later, in *Sardinops* there is a long history of publications documenting the fact that the "species" in different regions have no demonstrable differences. However, local scientific names continue to be used in most regions. In *Scomber* it is evident that there are only three species; *S. japonicus* which occurs in all of the major eastern and western boundary currents, *S. australasicus* which occurs in the Indo-Pacific (Kijima et al. 1986), and the temperate form *S. scombrus* which is found only in the North Atlantic (Collett and Nauen 1983). In *Engraulis* a separate species has been considered to occur in each current system and Nelson (1984) has suggested a separate subgenus for the forms in the California, Peru, and Falkland (Malvinas) currents. Also several taxonomic studies in progress suggest that *Engraulis* may be divided into two or three genera. In *Trachurus* the present taxonomic situation can only be described as confused, with papers that describe new species overlapping those that synonymize forms from widely dispersed areas of the world (Nekrasov 1978; Rytov and Razumovskaya 1984; Parrish 1989).

The question as to whether geographically isolated populations are distinct at the genus, species, subspecies, race, or fisheries stock level has significance far beyond the narrow range of taxonomy. If the distinctions among populations in widely separated current systems tend to be well below the species level, it is implied either that individual current systems are not closed systems for the species and that some exchange between current systems occurs or that the species has only recently (on a geological time scale) achieved its present geographical distribution. This would have great implications for research programs as diverse as evolutionary theory, zoogeography, ecosystem analysis, and fisheries management. In addition it would greatly enhance the value of interregional comparative analyses (Parrish et al. 1983).

In this work we have attempted to present the available taxonomic, genetic, geographical distribution, and stock structure information for one of these dominant and widespread ecotypes, the subtropical sardine, and to use this information to examine the degree of interchange which occurs among and within subtropical neritic regions of the world ocean.

Taxonomy

The Clupeidae are a difficult group to deal with taxonomically. Scores of genera and over 300 species have been described. A large proportion of these taxa has proved to be invalid and the status of other taxa is presently unresolved. One of the difficulties is that similar forms occurring in widely separated, but ecologically similar, regions of the world were originally described as separate species or even genera. As mentioned previously, local biologists continue to use old scientific names for many of these forms even after they have been shown to be indistinguishable from the forms in other regions. The sardines, or pilchards, are a good example of the present situation.

The North Atlantic–Mediterranean sardine, *Sardina pilchardus* (Walbaum 1792) was the first sardine to be described. It was originally described as *Clupea*, as were most of the *Sardinops* ecotypes described from 1842 to 1879. Valenciennes (1847) created the genus *Sardinella* to describe a tropical species of sardine, and for a few years some of the subtropical sardines were also referred to as *Sardinella*.

Gunther (1868) included all of the Clupeidae having a terminal mouth, minute or absent teeth, a complete midventral series of scutes, and an anal fin in the genus *Clupea*. Thus the subtropical sardines were included in the genus *Clupea* by most workers. Regan (1916, 1917), in his revisions of the Clupeidae, separated the sardines into a subtropical genus *Sardina* and a tropical genus *Sardinella*. Regan also examined sardines from all of the major subtropical areas of the world and decided that there were three species of *Sardina*:

1) *Sardina pilchardus*. With two subspecies; *S. p. pilchardus* off the Atlantic coast of Europe, from Portugal northwards to the British Isles, and *S. p. sardina* in the Mediterranean and off the Atlantic coast of North Africa.
2) *Sardina sagax*. With four widely separated populations; off the Pacific coast of South America, off the Pacific coast of North America, off Japan, and off South Africa.
3) *Sardina neopilchardus*. With populations off Australia and New Zealand and which Regan suggested was closely related to *S. sagax*.

Svetovidov (1952) in his extensive work on the Clupeidae repeatedly pointed out the close relationships between the "Clupeinea which migrate extensively and live mainly in the upper layers of the water (*Sardinops, Sardina, Sardinella*)." He notes the following unique characteristics which they share:

1) The dorsal fin is closer to the front of the body in *Sardina, Sardinops,* and *Sardinella*.
2) The lateral line canals on the head are most devloped in *Sardina, Sardinops,* and *Sardinella*.
3) The last two fin rays of the anal fin are elongated into lobes in *Sardina, Sardinops, Sardinella,* and *Clupeonella*.

4) Individuals have dark lateral pigment spots on the body in *Sardina, Sardinops* and some species of *Sardinella* and *Harengula*.
5) There are two elongated scales on the base of the lobes of the caudal fin in *Sardina, Sardinops,* and *Sardinella*.
6) There are transverse striations on the body scales which are interrupted in the center in *Sardina, Sardinops,* and *Sardinella* and unbroken in *Harengula*.
7) There are radial striations on the operculum of *Sardina* and *Sardinops*.

The major characteristics which separate the three genera are:

1) In *Sardina* the body scales are variable in size with smaller scales hidden underneath the larger scales.
2) In *Sardinops* the gill rakers on the lower part of the gill arch decrease in size toward the corner of the arch and the rakers on the upper part of the arch overlap the rakers on the lower part.
3) In *Sardinella* there are bilobed dermal outgrowths on the vertical portion of the cleithrum and there are no striations on the operculum.

Thompson (1926) in a morphological comparison of the European sardine (*S. p. pilchardus*) and the California sardine, which he called *Sardina sagax*, described a number of characteristics which could be used to separate the species. Hubbs (1929) used Thompson's results to establish a new genus, *Sardinops*, in which he included Regan's *Sardina sagax* and *Sardina neopilchardus*. His new genus was based on the six characteristics listed below:

1) In the American species there is usually a row of dark blotches behind the head, typically not apparent in the European.
2) The scales in the American species are arranged in a regular order, whereas the European are irregular with each alternating row not being nearly overlapped by the one in front.
3) The ventral scutes are weaker and less keeled, and have less expanded bases than the European species.
4) The gillrakers on the lower limb, unlike the European species, are shortened at the angle of the arch and they differ markedly in number at comparable sizes. In addition the upper limb folds down over the lower limb near the angle and there are differences in the minute processes on the gill rakers.
5) The interopercle is more expanded and widely exposed behind the preopercle.
6) The opercular ridges (and preopercle edge) are strongly oblique instead of being nearly vertical.

Hubbs (1929) examined *Sardinops* from California, Chile, Japan, and Australia and although he found no differences between these forms and even mentioned that "The distinctness of *Sardinops caerulea* is particularly doubtful," he differed from Regan (1916) and Thompson (1926), and used different scientific names for the populations in each of the major current systems in which the form occurs. Therefore the accepted usage for *Sardinops* became as follows:

1) *Sardinops sagax* (Jenyns, 1842)	Chilean
2) *Sardinops melanosticta* (Temminck & Schlegel, 1846)	Japanese
3) *Sardinops ocellata* (Pappe, 1853)	South African
4) *Sardinops caerulea* (Girard, 1854)	Californian
5) *Sardinops neopilchardus* (Steindachner, 1879)	Australian

A number of taxonomic studies have been carried out comparing various populations of *Sardinops*. Thompson (1926) found no significant differences in sardines from California, Japan, and Peru. Taranets (1938) was unable to find significant differences between the Japanese and California sardines and suggested that the two forms were subspecies. He also found that Japanese and California sardines had keel scales that were essentially the same as those in *Sardina*, thus invalidating one of the characteristics Hubbs (1929) used to create the genus *Sardinops*. Fowler (1941) agreed with Regan (1916) in including the South American, Californian, South African, and Japanese forms as a single species; however, he considered the use of the genus *Sardina* invalid and suggested that the proper name for all of the subtropical sardines was *Arengus*. The usage of the generic name *Arengus* was, however, not picked up by most authors. Amemiya and Tamura (1943) concluded that the degree of difference between the Japanese and California sardines was no more than typically occurs within a single species. Hildebrand (1946) examined *Sardinops* from Peru and California and found differences which he considered significant only at the subspecies level.

Svetovidov (1952) considered all of the populations of *Sardinops* to be the same species, *Sardinops sagax*. He also stated that *Sardina* often has lateral pigment spots and pointed out that this is a characteristic which it shares with *Sardinops* and *Sardinella*. This invalidated another characteristic which Hubbs (1929) used to establish the genus *Sardinops*.

De Buen (1958) examined sardines from Chile, Peru, and California and used the results of Thompson (1926) and Hildebrand (1946) to propose that there were three subspecies of sardines in the eastern Pacific:

1) *Sardinops sagax musica*	Chile
2) *Sardinops sagax sagax*	Peru
3) *Sardinops sagax caerulea*	California

However, Saez (1980) showed that there is no reason to distinguish between two subspecies for the Peru Current and based on meristic and morphometric evidence he concluded that the populations of sardines distributed along the coast from Ecuador to Chile comprise a single subspecies, *Sardinops sagax sagax*.

In contradiction to the studies listed above, species synopses for *Sardinops sagax* (De Buen 1960), *Sardinops caerulea* (Ahlstrom 1960), *Sardinops melanosticta* (Tokai Regional Fisheries Research Laboratory 1960), *Sardinops ocellata* (De Jager 1960; Matthews 1960), and *Sardinops neopilchardus* (Blackburn 1960) were presented at the *World Scientific Meeting on the Biology of Sardines and Related Species* which was held in Rome in 1959. The taxonomic sections of these species synopses did not contain a single piece of evidence suggesting that the various forms were indeed separate species and most of the synopses quoted one or more of the above works which state that differences between the various geographically isolated forms are too small to consider them as separate species. Nevertheless they were given their traditional species names; this appears to have been influenced by the fact that at that time a review of *Sardinops* was apparently underway at the Scripps Institution of Oceanography. Ahlstrom (1960) stated that conclusions then permissible from this study indicated that all cur-

rently recognized species would be synonymized under the name *Sardinops sagax*; only *neopilchardus* from Australia and New Zealand appeared to merit subspecific rank. This study, apparently by Hubbs, was never published and presently the traditional species names are used, with the exception that the California form is now considered to be *S. sagax* (Robins et al. 1980) and the Russians consider the Kuroshio form to be *S. sagax* (Kenya 1982).

Much of the differentiation of the Clupeidae has been associated with variations in the gill arches in association with adaptations to feeding (Nelson 1967). Nelson suggests that overlapping gill rakers, in some, and the mediopharyngobranchial in all alosins seems to place this subfamily at a higher level of specialization than that of the Clupeinae. Nelson points out that some of the more advanced Clupeinae (*Sardinops* and *Sardina*) have both overlapping gill rakers and a mediopharyngobranchial and that several others (*Sardinella* and *Opisthonema*) have a mediopharyngobranchial. Nelson also suggested that based on similarities in gill arch structure *Sardinops* and *Sardina* are closely related forms and probably should be recombined in a single genus. More recently Whitehead (1970) suggested that Svetovidov's solution (i.e. to recognize five subspecies of *Sardinops*) seemed to be the best.

The history of taxonomy in the sardines suggests that it is easier, and much more acceptable, to describe species than to synonymize them. In spite of the fact that no one has been able to find a characteristic feature with which to separate the various *Sardinops* populations, many workers dismiss contrary evidence such as Svetovidov's (1952) work with comments such as "There is evidence of a 'lumper' at work here!" (Culley 1971) and continue to use individual species names for each of the sardines in each major current system. The fact that the American Fisheries Society (1960) had accepted *Sardinops sagax* as the correct scientific name for the Pacific sardine for more than a decade before Culley's work was published also did not deter him from using the name *Sardinops caerulea* for the California population. Geographic distance rather than meristics, morphometrics or genetics appears to have been the major characteristic utilized in the definition of the presently accepted species of *Sardinops*.

Distribution

One of the most significant features of the sardines is their extensive anti-tropical distribution. *Sardinops*, or *Sardina*, occur in all of the eastern boundary currents and in the western boundary currents of the Indo-Pacific. When their population level within a current system is high they dominate the entire neritic zone of the current system from approximately the area of the west wind drift to the subtropical convergence. When their population level is low their distribution is greatly reduced and they are at times totally absent from areas which are productive fishing areas during periods of high abundance.

Canary Current

It is generally accepted that there are two subspecies of *Sardina* in the North Atlantic and Mediterranean. *Sardina pilchardus pilchardus* occurs off the Atlantic coast of Europe to southern Norway (59°N) (Riedel 1960) and *S.p. sardina* occurs in the Mediterranean and in the Atlantic off North Africa and off the Azores (Fig. 1). However, the distribution of *S.p. sardina* around and north of the British Isles appears to be associated with warm periods and increases in its' abundance (Demir and Southward 1974; Binet 1986; Southward and Boalch 1986). In the past *S.p. sardina* extended to as far south as Cap Blanc (21°N) but it was not abundant south of Cape Bojador (26°N); however, during the mid 1970's there was a large increase in sardines south of Cape Bojador and in association with this increase sardines were taken as far south as the Bay of Goree, Senegal (15°N) (Boely and Freon 1980).

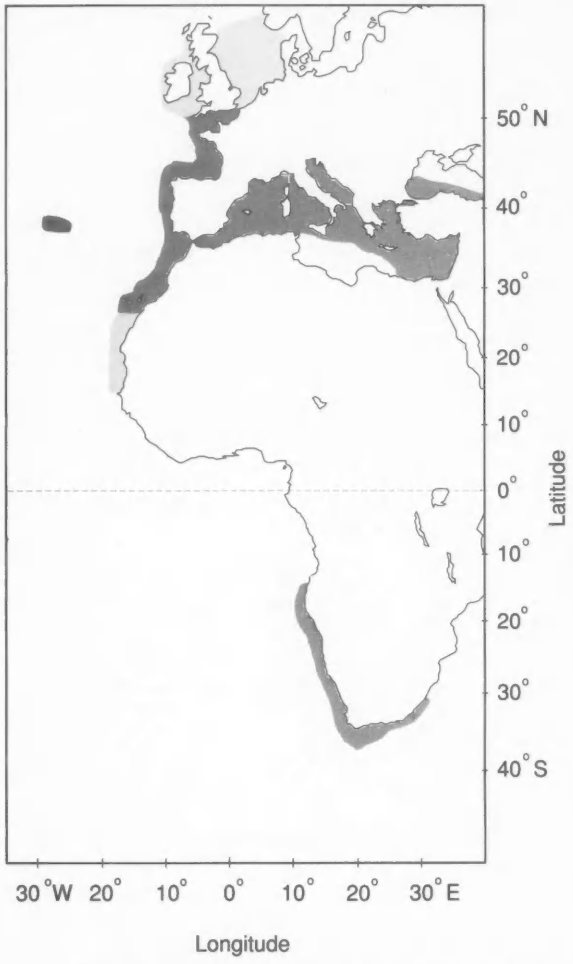

FIG. 1. Distribution of *Sardina* and *Sardinops* in the Atlantic at low abundance (dark shadiong) and the increase in range that occurs at high abundance (light shading).

It is difficult to assess the relative importance of spawning grounds due to the lack of synoptic, large-scale egg and larval surveys covering major segments of *Sardina's* range; however, spawning takes place over a wide portion of *Sardina's* range. In the European Atlantic region spawning appears to be concentrated in the northern Bay of Biscay and the western side of the English Channel (Demir and Southward 1974). Franco and Lago de Lanzas (1976) report spawning grounds off the Cantabric coast in northern Spain, and Atunez, et al. (1973) report

spawning off central Portugal. In the Mediterranean "junior stages are found in all sardine areas," according to Larranetta (1960), who lists a large number of spawning areas where young stages are at high concentrations.

Bravo de Laguna et al. (1976), analyzed seasonal migration and maturity stages, and suggest that in the Moroccan area the principal *Sardina* spawning occurs between Cap Juby (28°N) and Agadir (30°N). Little evidence is available for the Saharian region; however, spawning has been reported in the region (Domanewsky and Barkova 1976).

Benguela Current

According to Davies (1956) the range of *Sardinops* in southern Africa extends from Baia dos Tigres, Angola (16°S) around the Cape of Good Hope (35°S) to Durban, South Africa (30°S) (Fig. 1). Spawning grounds are located in the area between Cape Frio (19°S) and Walvis Bay (24°S) (King 1977a) and on the Agulhas Bank (35°S); however, before the decline in landings in the latter area, older sardines also spawned in the area west of St. Helena Bay (32°S) (Shelton 1981).

California Current

In the California Current system *Sardinops* occurs from southern Baja California (23°N) to southeastern Alaska (57°N) and in the Gulf of California (Fig. 2). During the peak in the fishery in the 1930's annual catches in excess of 100 000 tons were taken in Canada; however, with the total collapse of the fishery in the 1950's and early 1960's the range decreased markedly and sardines were virtually absent from the area north of Point Conception (34°N) from the mid-1960's until the early 1980's when a minor recovery of the population resulted in the occurrence of sardines as far north as San Francisco (38°N). Spawning grounds are located from the Southern California Bight (34°N) to southern Baja California (23°N) (Kramer 1970) and in the central portion of the Gulf of California (26–29°N) (Sokolov 1974).

Peru Current

The early literature gives the distribution of *Sardinops* in the Peru Current region from Secchura, Peru (5°S), and around the Galapagos Islands (Hildebrand 1946; Alverson and Shimada 1957) to the Gulf of Arauco (37°S) in Chile (Delfin 1900; Mann 1954). Other authors point out that sardine did not extend south of Coquimbo (30°S) during the late 1950's and early 1960's (De Buen 1958; Brandhorst 1963). During the 1970's sardine abundance increased in the Peru Current region and its range now extends from the equator (0°S) in Ecuador (Garcia 1983) to Chiloe (42°S) in Chile (Serra 1986) (Fig. 2). According to Santander and Flores (1983) spawning grounds off Peru are centered at 5–12°S. Off northern Chile, spawning is centered at 18–26°S and a separate spawning area now occurs from 34–39°S, near Talcahuano (Serra 1983). Paleosediment analyses also suggest that sardines have had population centers in northern Peru (7–10°S) and northern Chile (17–22°S) and a population minimum in central and southern Peru (11–16°S) during much of the Holocene (DeVries and Pearcy 1982).

Kuroshio Current

In the Kuroshio Current region *Sardinops* occurs from south of Japan (29°N) to the east coast of Kamtchatka (54°N) (Tokai Regional Fisheries Research Laboratory 1960) (Fig. 3). During the 1930's when population levels were high the spawning grounds of the Japanese sardine were concentrated in the area around southern Kyushu (29–33°N); sardines from this area migrated northwards throughout the Sea of Japan, along the Pacific coast of Japan to Hokkaido and through the Tsugaru Straits (Nakai 1952). During the 1940's sardines markedly declined in the landings and the fishery was greatly reduced in the northern portion of its range around northern Honshu and Hokkaido, being primarily restricted to the western Sea of Japan and East China Sea; spawning was concentrated in the Sea of Japan northwest of Kyushu (33–35°N) (Nakai 1960). The fishery recovered during the late 1970's and the recovery was associated with recolonization of the spawning grounds around southern Kyushu, a major increase in spawning grounds on the Pacific Coast of Honshu (30–36°N) and the redevelopment of the fishery in northern Honshu and Hokkaido (Tanaka 1983; Watanabe 1983). During the recent period of high abundance, sardines were taken in the Kuroshio Extension at 171°E,

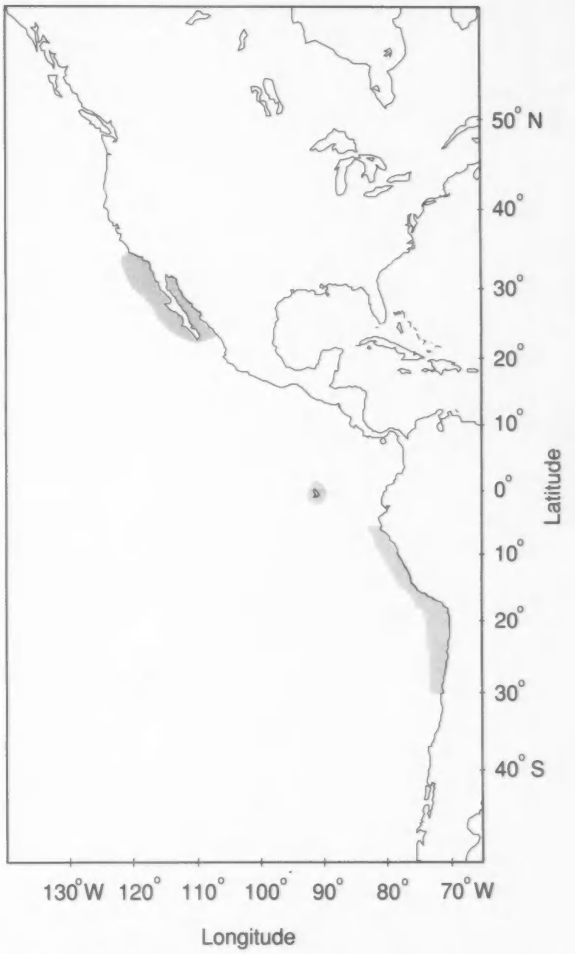

FIG. 2. Distribution of *Sardinops* in the eastern Pacific at low bundance (dark shading) and the increase in range that occurs at high abundance (light shading).

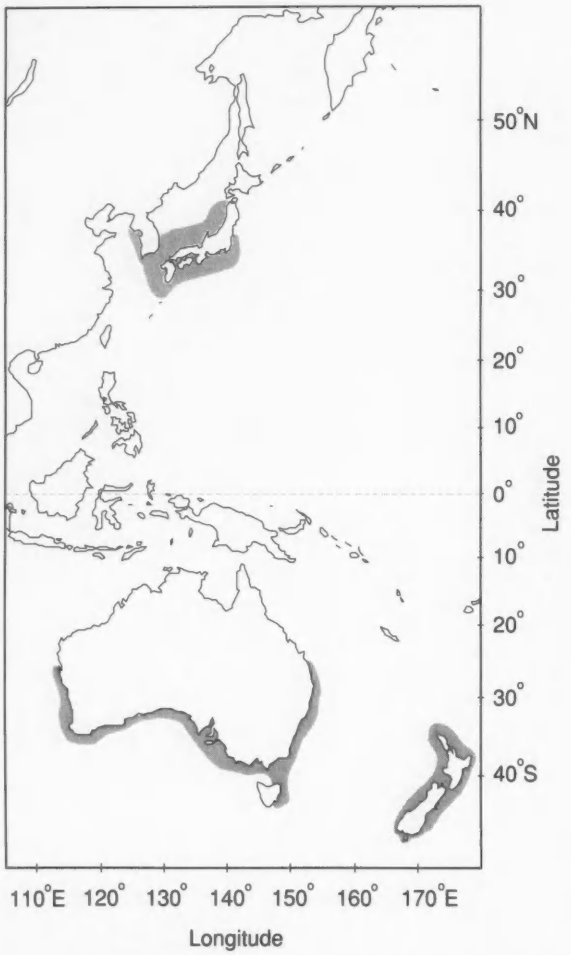

FIG. 3. Distribution of *Sardinops* in the Indo-Pacific at low abundance (dark shading) and the increase in range that occurs at high abundance (light shading).

approximately one third (2800 km) of the way across the Pacific (Kenya 1982).

Australia and New Zealand

Much less information is available on sardine distribution and its variability in Australia and New Zealand than in the other systems due to the lack of a significant fishery. Blackburn (1960) reported the northern limits of the range of sardines in Australia to be 24°S on the west coast and 25°S on the east coast. Their range extends south to southern Tasmania (43°S) and Baker (1972) reported their distribution from 34°S to 46°S in New Zealand (Fig. 3). No extensive egg and larval surveys have been carried out in the Australian and New Zealand region and the distribution of spawning of sardines in the region is not well known; however, Blackburn (1960) shows the breeding range nearly equaling the geographical range in Australia, whereas in New Zealand spawning is concentrated in the area between the North and South Islands near 40°S.

Changes in Geographical Sardine Distribution

The observed variations in the geographical distribution of sardine stocks associated with changes in population size have a similar pattern in each of the current systems where sardines have been heavily exploited (and therefore extensively studied). Sardine spawning grounds have been abandoned as populations decreased in size, or colonized as population size increased, or both. In the Canary Current increases in population size of the Sahara sardine stock was associated with the establishment of a "new stock" in Senegal (Freon and Stequert 1979). The higher latitude distribution of sardines in the Atlantic, i.e. in the English Channel, occurs in association with increased population size (Binet 1986; Southward and Boalch 1986). In the Peru Current increases in the population size in Peru and northern Chile were associated with records of spawning as far north as the equator and with colonization of 'new' spawning grounds in the Talcahauno, Chile region (Garcia 1983; Serra 1983). In the California Current an extreme decrease in the population size resulted in the abandonment of most of the northern range of the California stock. In the Kuroshio Current sardines abandoned much of their range and spawning grounds when the population declined and then several years later recolonized much of their former range and spawning grounds as the population size increased; however, the geographical distribution of biomass and the relative importance of different spawning grounds during the population resurgence was considerably different from that of the previous population maximum (Kondo 1980; Watanabe 1983). The present (1987) spawning range and geographical distribution has returned to very nearly that of the 1930's (T. Kawasaki, Tohoku Univ. Sendai, Japan, pers. comm.).

An outstanding feature of *Sardinops* is the similarity in the timing of extremely large, multi-decade population fluctuations occurring in the Kuroshio, Peru and California current systems (Shuntov and Vasilkov 1982; Kawasaki 1983). As shown above, these fluctuations also resulted in extensive alterations in the geographical distribution of *Sardinops* in each system.

Stock Structure

The stock concept has been interpreted in several different ways in marine fishes. The concept had its inception in studies which demonstrated morphological differences between geographic areas and has become an important organizing principle in the design of research and formulation of management policies by fishery agencies (Cushing 1968). With the realization that morphological variation may merely reflect the response to geographic variation in environmental conditions, and with the development of biochemical methods capable of detecting Mendelian variation, considerable effort has been directed toward using genetic methods, such as protein electrophoresis, to search for discrete stocks (e.g. Allendorf and Utter 1979; Ihssen et al. 1981). The rationale for using genetic methods was that stocks were thought to be reproductively isolated from each other to some degree by distance or by barriers to migration such as warm or cold current systems. Reproductive isolation between stocks is thought to lead to genetic differentiation which is potentially detectable by biochemical methods.

The term "stock" is used differently by different workers. It has been used by some to designate a biologically significant unit; others use the term merely to designate artificial subdivisions of fish into geographic or political areas. For purposes

of this review we will use the term stock in its biological sense as an extension of the genetic concept of a population; that is as an aggregation of fish which may or may not be genetically discrete from other aggregations of fish, but one which is isolated, on at least a decadal time scale, from other aggregations.

In each of the major current systems in which sardines occur it is presently believed that there are several stocks. This belief is partially due to the presence of several geographically separated spawning grounds; however, in many cases stocks have been identified by differences in meristic or morphological characteristics such as the number of vertebrae, the number of gill rakers, or differences in body proportions. In most cases these differences appear to be small enough so that it is impossible to determine whether they are caused by developmental responses to variations in the environmental conditions present in the different spawning and nursery grounds or by differences in gene pools. As will be demonstrated later, sardines from high or low latitude regions of different current systems often tend to be more alike in these characteristics than are sardines from high and low latitude areas within a current system. This is not uncommon as a negative correlation between the number of vertebrae and the temperature of their habitat has been found in a number of Clupeids (Muzinic 1960). It also should be noted that it is not possible to use average values such as those derived from meristic or morphometric characteristics to determine the degree of annual admixture between the populations in different spawning areas. In addition, in a few of the current systems some electrophoretic and serological studies of sardine stocks are available.

The following sections review the current knowledge of the stock structure in the regions in which *Sardina* or *Sardinops* occur.

Canary Current

Sardina pilchardus is the most studied of the sardines; an extensive review of the geographical variations in its meristic and morphometric characteristics has been published by Freon and Stequert (1979). The two currently accepted subspecies, which are based parimarily on the number of gill rakers, both occur in the Atlantic. *Sardina pilchardus pilchardus* is generally considered to occur off western Europe, and *S.p. sardina* occurs off North Africa and in the Mediterranean. Determination of the stock structure within the Mediterranean appears to be quite controversial, based on quite small scale geographical features, and a solution was beyond the language skills and endurance of the present authors. In the Atlantic there appears to be some consensus on stock structure.

Bravo de Laguna (1983) points out that *Sardina* extends from Dogger Bank in the North Sea to Cap Vert in Senegal. In this large area Andreu (1969) and Furnestin and Furnestin (1970) distinguished the following geographical stocks.

1) The septentrional Atlantic stock which occurs from the North Sea (57° N) to the Cantabric coast of Spain (43°N). (Vertebrae 52.0)[1]
2) The Iberian or meridional Atlantic stock which occurs from the Cantabric coast to the Straits of Gibraltar (36°N). (Vertebrae 51.2)
3) The Moroccan stock which occurs from Cap Spartel (36°N) to Cap Juby (28°N). (Vertebrae 51.0)
4) The Saharian stock which occurs from Cap Juby (28°N) to Levrier Bay (21°N). (Vertebrae 50.3)

On the basis of the number of vertebrae, electrophoretic differences, and growth differences it has also been suggested that three stocks exist off North Africa (Anon. 1978):

1) A stock near Casablanca (34°N).
2) A stock between Safi (32°N) and Cap d'Anlou (30°N).
3) A stock between Ifni (29°N) and El Ayoun (27°N).

Bravo de Laguna (1983) analysed the results of hydroacoustic surveys and seasonal migrations of sardines off North Africa and concluded that sardines between Safi and El Ayoun might belong to the same stock. He also stated that there was not enough evidence on sardines south of Cap Bojador (26°N) to assess the stock structure in this region.

Analyses of all of the information available on biometric and meristic characters, electrophoretic studies, spawning areas and periods of reproduction, growth studies and distributions observed during hydroacoustic surveys have recently led several workers to suggest that there are three stocks off North Africa (Belveze and Erzini 1983; Rami 1984):

1) Casablanca stock (36°N to 33° 30′N).
2) Moroccan stock (32° 30′N to 27°N).
3) Saharian stock (26°N to 21°N).

Beginning in about 1970 there were increasing amounts of sardines caught along the coast of Mauritania between the latitudes of about 16°N to 26°N and since about 1974 sardines have been taken as far south as Senegal (Anon. 1978). Freon and Stequert (1979) present very convincing evidence that the expansion of the population has resulted in a new stock off Senegal. They also present evidence which places the separation of *Sardina pilchardus* into two subspecies based on the number of gill rakers or vertebrae in doubt. Their fig. 8, which shows the relationships between the number of gill rakers and total length for sardines taken at a wide variety of locations, clearly demonstrates that although there is a strong tendency for the Atlantic, European sardines to have a larger number of gill rakers than the Atlantic, North African and Mediterranean sardines, this is not always the case. For example, sardines taken near Casablanca which are supposedly *S.p. sardina*, the subspecies with the smaller numbers of gill rakers, actually have the largest number of gill rakers of any of the locations. Sardines from their proposed Senegalese stock also have very large numbers of gill rakers. The number of vertebrae appears to be a good characteristic in separating the two stocks of *S.p. pilchardus*; however, the number of vertebrae in *S.p. sardina* taken in most of the Mediterranean areas are intermediate between the two *S.p. pilchardus* stocks and the rest are similar to the Iberian stock or have even fewer vertebrae.

In summary, there appears to be at least four stocks of *Sardina pilchardus* in the northeastern Atlantic. The most likely expansions from the four stock descriptions of Andreu (1969) and Furnestin and Furnestin (1970) given above are the possibility of two Moroccan stocks as suggested by Belveze and Erzini (1983) and Rami (1984), the possibility of a Senegalese stock which is separate from the Saharian stock as suggested by Freon and Stequert (1979) and a stock in the Azores.

[1]Vertebrae counts were made in different ways by different authors. We have standardized the counts to that suggested by Hubbs and Lagler (1947), (ie. the hypural plate is counted as one vertebrae).

Benguela Current

On the west coast of southern Africa, the Benguela upwelling system may act to some degree as a barrier to migration between sardines off Namibia and those of the southern portion of South Africa. The region of maximum upwelling, which lies between the two regions, may reinforce reproductive isolation between the two regions as it has temperatures which are below the minimal temperature needed for normal sardine larval development (King 1977b). Davies (1956) suggested that there was only a single stock based on small regional differences in the numbers of verbebrae. Newman (1970) concluded, on the basis of tagging experiments, that there was little exchange of individuals between the Walvis Bay fishery in Namibia and the Cape fishery in South Africa. The presence of two widely separated spawning grounds, sharp differences in historical landings in the two areas, and Newman's work led to the general acceptance of the two-stock concept in southern Africa (Butterworth 1983; Crawford et al. 1983):

1) Cape stock (34°S). (Vertebrae 51.2 to 51.4)[2]
2) Walvis Bay stock (22°S).

These stocks have been the subject of three genetic studies using electrophoretic methods. Thompson and Mostert (1974) examined three samples extending from Namibia to the west coast of South Africa for geographic variation at a single locus encoding an esterase enzyme. Allele frequency differences in that study suggested that regional genetic differences may exist between the two areas. Le Clus (1978) searched for geographic variation among five samples taken from the Namibian stock and also detected a significant degree of heterogeneity. In both of these studies, however, there were significant departures from Hardy–Weinberg proportions; these could have resulted from three effects. The first is that the samples may have included individuals from two or more genetically differentiated stocks (the Wahlund effect). The second is that an undetected null or silent allele was present which produced the apparent excesses of homozygotes in the samples. The third is that the phenotypic variation may not have been under simple genetic control. Spurious electrophoretic bands can appear through storage and handling effects (Utter et al. 1979). In the latter two cases any attempt to infer genetic differences between stocks using the data is invalid.

In the third study, Grant (1985b), examined the variation in 30 protein coding loci for six samples extending from 19°N in northern Namibia to 26°S on the south coast of South Africa. Four proteins (excluding esterase) were sufficiently polymorphic to make statistical tests of geographic structure, but no significant differences in allele frequencies were detected among the samples. A gene diversity analysis (Nei 1973) indicated that 99.5% of the total genetic variation that was detected in the samples was contained, on average, within each location. Only 0.5% of the total variation was due to allele-frequency differention among the locations that were sampled. The lack of regional differentiation implies either that there is sufficient migration between the stocks to prevent genetic differentiation or that reproductive isolation between the stocks has occurred only recently, and insufficient time has elapsed for significant differences in allele frequencies to appear. The former explanation is more likely.

California Current

It has been generally accepted that there are at least three stocks of *Sardinops* in the California Current system and a fourth has been proposed (Radovich 1981). Clark (1947) used vertebral counts to demonstrate that sardines in Baja California differed from those further north. Clark and Janssen (1945) and Janssen (1948) used tagging studies to show that there was significant intermixing of sardines between northern Baja California and California and between California, Oregon, Washington and British Columbia. McHugh (1950) found morphometric and meristic differences between sardines in southern Baja California and those to the north. Vrooman (1964) separated the California Current sardines into the following three subpopulations based on serological differences, and he also noted that the number of vertebrae differed between the three subpopulations:

1) Northern stock, from northern Baja California (30°N) to Alaska (55°N). (Vertebrae, 51.44)
2) Southern stock, off the Pacific coast of Baja California (23–30°N). (Vertebrae, 51.36)
3) Gulf of California stock (23–31°N). (Vertebrae, 51.04)

The ranges of the southern and northern stocks appear to overlap seasonally by at least 400 km; southern stock fish were taken as far north as San Pedro, California (34°N) and northern fish were taken as far south as San Quintin Bay, Baja California (30°N); however, it appears that both groups move north or south at about the same time of year with no overlap of the stocks (Vrooman 1964).

In contradiction to Vrooman (1964) recent morphological and biochemical genetic studies have not revealed genetic variation between sardines from central California, the Pacific side of southern Baja California and the Gulf of California (Hedgecock et al. 1985).

Radovich (1962, 1981) postulated that the sardines off the Pacific Northwest (Oregon to Canada) and southern California were somewhat distinct, either genetically or due to a strong tendency for fish to favor areas in which they were born. He pointed out that sardines in the Pacific Northwest were larger and older than those in southern California and that there were differences in the growth patterns on scales from fish in the two areas. The occasional presence of spawning and occurrences of young-of-the-year sardines in the Pacific Northwest (Walford and Mosher 1941) also gives some credence to separation of sardines in the southern California and Pacific Northwest areas. However, the relatively small proportion of young fish in the Pacific Northwest fishery (Radovich 1981) and the rapid movement of tagged fish between the Pacific Northwest and southern California can be used to argue against a separate far northern stock (Janssen 1938). Marr (1960) and Murphy (1966) both rejected the existence of a far northern stock.

Peru Current

The stock structure of sardines in the Peru current system is presently no better understood than that of the other systems and it is further complicated by the recent high population levels which have resulted in a geographical expansion of the range and also apparent recolonization of spawning grounds which

[2]Average vertebrae counts for the Cape stock were given by Baird (1971) as 49.2–49.4. According to P. Shelton (Sea Fisheries Institute, Capetown, South Africa) these counts did not include the urostyle or hyperal plate; for comparative purposes we have therefore increased the counts by two.

were not occupied in the decade prior to the population expansion. There is no conclusive evidence regarding the fishery stock units but there could be three or possibly five stocks based on the spacial pattern of spawning:

1) Northern stock(s), from southern Ecuador (1°S) to central Peru (15°S). The Galapagos Islands may have a separate stock.
2) Central stock, from southern Peru (15°S) to northern Chile (25°S). (Vertebrae 50.87)
3) Southern stock(s), off Coquimbo, Chile (30°S). (Vertebrae 51.15) Off Talcahuano, Chile (37°S).

Supporting evidence for the stock structure of sardines in the Peru Current system is quite limited. Hildebrand (1946) found differences in the number of dorsal rays, 17–20 in Peru vs 15–18 in Chile, and in the relative length of the head, 3.3–3.7 head lengths is the standard length in Peru and 3.48 in Chile. De Buen (1958) gave the following differences between the sardines in Peru and Chile; length of the head 27–28.5% of the standard length in Peru and 25.5–26.1% in Chile, predorsal distance 47–50% of standard length in Peru and 45.1–47.6% in Chile.

Brandhorst et al. (1965) analyzed sardines from Arica, Chile (18°S) and from Coquimbo, Chile (30°S). They found that the mean number of vertebrae were 50.874 ± 0.034 at Arica and 51.153 ± 0.070 at Coquimbo suggesting stock differences may occur between these two regions. The recent expansion of sardines into the Talcahuano region of Chile (37°S) probably occurred from sardines in the Coquimbo region (30°S). The presence of spawning at this new location may indicate that the populations of sardines in these two regions are at least partially independent.

Another feature in the Peru Current is that large geographical dislocations of sardines have been observed in association with El Niño events. Before 1983 the main concentrations of sardines were in northern and central Peru and in northern Chile (Zuta et al. 1983; Serra 1983); however, during the first quarter of the El Niño year of 1983 there was an influx of sardines into southern Peru. It is assumed that these fish were primarily from northern and central Peru, but it is possible that fish from northern Chile also moved into southern Peru as suggested by tag recoveries and spacial distributions shown by hydroacoustic surveys. The Ecuadorian sardine fishery suffered a sharp decline as sardines migrated south into Peruvian waters, apparently avoiding the high water temperatures caused by the same El Niño event (Anon. 1984). Evidence from "normal" years suggests that the abundance of sardines in northern Chile shows a seasonal pattern with peak abundance occurring during the winter spawning season and much lower abundance during the summer and fall (Serra and Tsukayama 1988).

Tagging experiments have shown that during the 1982–83 El Niño there were movements of sardines from both northern and central Peru into Chile (Torres et al. 1984). Maximum movement observed was from approximately 9°S to 30°S (over 2000 km). In other years, tag recoveries suggest that fish move seasonally to and from northern and central Chile (i.e., from 18°S to 30°S) with southward recoveries at a maximum during the summer and northward recoveries at a maximum during the winter.

When the available evidence is viewed as a whole, it is obvious that there is considerable evidence for intermixing of fish from central Peru to Coquimbo, Chile. It is likely that definitive analysis of the stock structure in the Peru Current will only be possible when properly designed biochemical genetic analyses have been carried out.

Kuroshio Current

The number of sardine stocks in the waters associated with the Kuroshio Current system has been the subject of considerable debate. The number of stocks proposed to inhabit the area has varied from one (Nakai 1952, 1962) to five or seven (Uda 1960).

According to Nakai (1952), during the period of high abundance in the 1930's the spawning grounds of the Japanese sardine were heavily concentrated in the area around southern Japan (29–33°N); sardines from this area migrated northwards throughout the Sea of Japan, along the Pacific coast of Japan and through the Tsugaru straits. Based on this information he suggested that there was only one stock.

According to Uda (1960), Aikawa and Konisi (1940) considered that there were two stocks.

1) Sea of Japan stock. (Vertebrae 51.75 ± 0.45)
2) Pacific stock. (Vertebrae 51.46 ± 0.55)

Based on the time sequence of catch data, Uda (1960) divided the population into five or seven principal groups for which he suggested that there were spawning grounds corresponding to the various groups but he did not identify the locations.

According to Kondo (1980), after the population size declined in the 1940's it became apparent that there were four subpopulations of the Japanese sardine; however, Kondo presents no documentation for these four subpopulations:

1) Kyushu subpopulation: Spawns along the Western coast of southern Japan) Kyushu to San-in district) and occupies the area from the East China Sea to the Western part of the Sea of Japan.
2) Japan Sea subpopulation: Spawns around the Noto Peninsula (Ishikawa Prefecture) and occupies all of the Sea of Japan.
3) Ashizuri subpopulation: Spawns along the Pacific coast of southern Japan (Kyushu and Shikoku).
4) Pacific subpopulation: Spawns in the Pacific waters along Honshu.

Watanabe (1983) stated that there are two subpopulations on the Pacific side of Japan. However, his statements suggest that the Japanese perspective of subpopulations is quite different from the concept of stock used in this review. For example, he stated that it is assumed that one of the major reasons for the burst in the spawning in the Ashizuri subpopulation was the substantial immigration of parental fish, especially the 1977 and 1978 year-classes from the Pacific subpopulation. He further stated that it is apparent that a substantial amount of the larvae of the Ashizuri subpopulation have been transferred and therefore recruited to the Pacific subpopulation and that intermingling of juvenile and adult fish has widely taken place between the two subpopulations after the expansion of the distribution range in each.

We were unable to find evidence from tagging, biochemical analyses, or traditional taxonomic studies on which to base an analysis of stock structure in the Japanese region. However, the available literature suggests considerable intermixing of sardines throughout the Kuroshio Current region, particularly when abundance is high.

Australia and New Zealand

Blackburn (1960) suggested that two well-separated winter spawning and two well-separated summer spawning regions occur in Australia. On "the assumption that sardines spawn only once a year for a period of a few months only" he proposed that there were four reproductively isolated populations in eastern Australia, possibly two in western Australia and one in New Zealand. In view of recent evidence on spawning frequency of sardines (Alarcon et al. 1984) and the fact that spawning has been observed for extended periods in sardine populations from many areas of the world, our interpretation of Blackburn's (1960) data is that there are four stocks with spawning grounds in the following areas:

1) Western Australia, 27–35°S. (Vertebrae 50.86)[3]
2) Southern Queensland to southern New South Wales, 27–36°S. (Vertebrae 50.89)
3) Victoria, Tasmania, and South Australia, 34–41°S. (Vertebrae 50.83)
4) New Zealand, 35–46°S. (Vertebrae 51.39)

Discussion of Stock Structure

The concept that populations of fishes are subdivided into discrete stocks has a substantial appeal to those responsible for fishery management. Stocks of various species of fishes have been defined by geographic differences in meristic and morphological characters, by regional differences in population parameters, by physiology and behavior, by the results of tagging experiments and by physical oceanographic features (Ihssen et al. 1981; Iles and Sinclair 1982). With the development of such molecular methods as protein electrophoresis and the restriction enzyme analysis of DNA, it became possible to search for genetic differences between the putative stocks which had been defined by non-genetic methods. In the genetic stock concept, stocks are viewed as collections of individuals of a species which freely interbreed. In reality, individuals in the same species do not always freely interbreed because factors external to their genetic systems prevent them from physically mixing. The most important of these are isolation by geographic distance and physical barriers to migration, both of which can lead to some degree of reproductive isolation between local groups of fish. This reproductive isolation may lead to subsequent genetic divergence through natural selection or genetic drift.

The degree to which genetic differentiation among populations is realized varies according to the life history patterns which affect migration and gene flow among populations. Theoretical studies have shown that only a small amount of gene flow is sufficient to prevent genetic differentiation through genetic drift between populations (Wright 1940; Kimura 1955). Populations of freshwater fishes are isolated by physical barriers to a much greater degree than marine fishes. For instance, in freshwater fishes inhabiting lakes or geographically isolated river systems, the amount of the total genetic variation in a species which is due to population differentiation generally ranges between 15 and 20% (Gyllensten 1985). The remaining intraspecific diversity is contained within populations. In anadromous fishes, such as salmon and sea run populations of trouts, between 5 and 10% of the total genetic variation is due to differences between populations (Ryman 1983). Anadromous fishes are less restrained by barriers to migration than are purely freshwater fishes and thus show a greater degree of similarity between populations.

Marine fishes show an even greater degree of similarity between populations because of the large potential for gene flow through unrestrained adult migration or passive drift at the egg and larval stages. In a study of 10 species of near-shore fishes located along the Pacific coast of California and Mexico which had different life-history patterns, Waples (1987) found a negative correlation between dispersal capability and the amount of genetic differentiation among populations. On average, only 4% of the total genetic variation in a species was due to genetic differences among populations. One exception in Waples' study was a species lacking a pelagic larval stage for which 56% of its total variation was due to population differences. In other studies of near shore fishes which spawn benthic eggs less than 2% of the total variation over large areas (1000–3000 km) was due to regional differences (Atlantic herring (*Clupea harengus*) Grant 1984, Ryman et al. 1984; and Pacific herring (*C. pallasi*) Grant and Utter 1985).

Epipelagic or benthic marine fishes which spawn planktonic eggs into coastal current systems show an even greater degree of similarity between populations. Anchovies (*Engraulis*, Grant 1985b), Atlantic mackerel (*Scomber*, Jamieson and Smith 1987), hake (*Merluccius*, Grant et al. 1987), deepwater snapper (*Pristipomoides*, Shaklee and Samollow 1984), damselfish (*Stegastes*, Shaklee 1984), to mention a few neritic marine fishes that have been studied with genetic methods, typically exhibit virtually no genetic differences among locations. Usually much less than 2% of their total genetic diversity is due to regional differences. All of these results clearly demonstrate the homogenizing effect that gene flow has on the genetic stock structure of marine fish populations over large areas.

Grant et al. (1987) have further argued that genetic differences among stocks of marine fishes are unlikely to appear over short periods, on an evolutionary time scale, because genetic drift is unimportant in very large populations. Even for populations which become completely isolated from one another, at least N generations (where N = population size) must pass before any substantial divergence is likely (Kimura 1955). Since the population sizes in the dominant marine fishes tend to be very large, on the order of at least $N = 10^7$ individuals, genetic drift is not an important differentiating force.

Both the foregoing theoretical considerations and previous attempts to find discrete genetic stocks in marine fishes in general, and in southern African sardines in particular, suggest that it is unlikely that sardines within the individual current systems are subdivided into genetically definable stocks.

Speciation

One of the intriguing aspects of the subtropical sardines (*Sardina* and *Sardinops*) is that over much of their range they have few marine Clupeid competitors. In fact, the only other marine Clupeid genus which can be classified as subtropical is *Brevoortia* which occurs in the subtropical northwestern and southwestern Atlantic, the only subtropical, neritic systems in which sardines do not occur. However, the tropical sardine (*Sardinella*) co-occurs with *Sardina* off northwestern Africa and in the Mediterranean.

The subtropical regions are not the only ones with limited Clupeid faunas. There are only two marine sub-polar clupeid

[3]The number of vertebrae given one higher than that reported by Blackburn (1960) as he did not count the hypural plate.

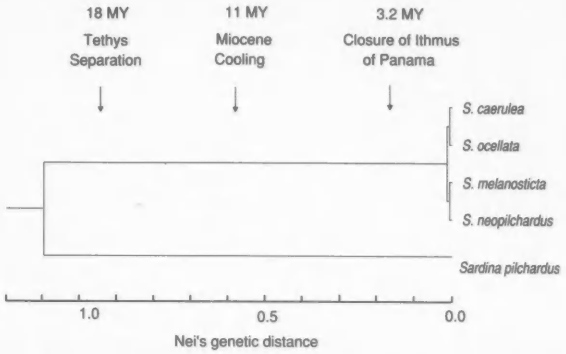

FIG. 4. A UPGMA cluster analysis of Nei's genetic distances (Nei 1972) between geographically isolated populations of *Sardina* and *Sardinops*. Genetic distances are based on data for 29 protein coding loci. Positions of paleo-events are based on an assumed relationship of 19 million years per 1 unit of Nei's genetic distance.

genera, *Clupea* and *Sprattus*, and these have very different distributions, co-occurring only in the northeastern Atlantic and the subpolar portion of South America. We note that according to Whitehead (1964) the taxonomic status of *Clupea bentincki* from South America is still not well resolved. There are, of course, a number of estuarine or anadromous Clupeid populations which are restricted to subtropical and subpolar regions (i.e. *Alosa, Pomolobus, Ramnogaster,* and *Clupeonella*).

Sardinops is unique in that the ecotypes which occur in widely separated current systems exhibit very limited meristic or morphometric diversification, the meristic variations of stocks within the various current systems have a similar latitudinal pattern, and each current system is considered to contain a number of fishery stocks which collectively comprise a very large biomass and occupy a huge geographical area.

It is possible, of course, that the forms in the various current systems look alike but have considerable genetic diversification at the physiological level. Recent biochemical genetic analyses (Grant and Leslie, unpubl. data), however, suggest that the morphological similarity between taxa appears to result from an underlying genetic similarity and not from convergent evolution in response to similar selective regimens in the various current systems.

Grant and Leslie analyzed tissue samples of four of the nominal species of sardines from Japan (*Sardinops melanosticta*), Australia, (*Sardinops neopilchardus*), California (*Sardinops caerula*), southern Africa (*Sardinops ocellata*), and a single species of sardine from Europe (*Sardina pilchardus*). Standard starch gel electrophoretic methods were used to analyze the gene products of 29 enzyme encoding loci. The Mendelian natures of the banding patterns on the gels were inferred according to the criteria outlined in Allendorf and Utter (1979). Allele frequencies were calculated from the resulting genotypic data and used to calculate Nei's (1972) genetic distances between each pair of taxa. Phenetic relationships among the taxa were then inferred using the unpaired group method of cluster analysis (UPGMA) (Fig. 4).

There are two important features of these results. The first is that there is a large genetic distance between the European sardine and the remaining taxa of sardines. The average genetic distance between the European sardine and the remaining sardines was 1.10, a value that is typical of genetic distances between well-differentiated genera in the same family (Thorpe 1982). This confirms the taxonomic separation of the European sardine (*Sardina*) from all of the remaining sardine taxa (*Sardinops*). The second important feature of these results is that there is very little genetic differentiation among taxa in the genus *Sardinops* even though they are separated by very large geographic distances. Genetic distances between the taxa represented by the four samples ranged from 0.002 to 0.009 and are more typical of genetic distances between conspecific populations rather than between species or even between subspecies (Thorpe 1982).

Other than the major division between *Sardina* and *Sardinops*, the cluster analysis of genetic distances did not reveal any apparently meaningful relationships among these taxa. The relationships among the four nominal species of *Sardinops* shown in the phenogram are most likely due to sample effects of the allele frequency data and cannot be taken as reflecting true biogeographic or phylogenetic relationships. Thus the lack of anatomical, morphometric and meristic differences between the *Sardinops* populations in widely dispersed regions of the world is reinforced by a corresponding lack of differences at the biochemical level.

We therefore conclude that *Sardinops* is a monotypic genus as proposed by Svetovidov (1952), and that *Sardinops sagax* is the correct scientific name for the populations in the Agulhas, Benguela, California, Kuroshio, and Peru Currents as well as for the Australian and New Zealand populations. The type specimen is *Clupea sagax* Jenyns, from Darwin's collections, and the description of the species is the original description (Jenyns 1847) plus the material presented by Hubbs (1929) in his description of the genus. *Sardinops melanosticta* (Temminck & Schlegel), *Sardinops ocellata* (Pappe), *Sardinops caerula* (Girard), and *Sardinops neopilchardus* (Steindachner) become synonyms of *Sardinops sagax* (Jenyns).

Biogeography

Discussions of biogeography and speciation are heavily dependent upon the biases of the individuals concerned and before proceeding further we will state our biases in regard to the biogeographic principles under which we are interpreting the sardine data. Firstly, we assume that speciation occurs predominantly as a result of isolation in allopatry (Futuyma and Mayer 1980). This is especially true for epipelagic fishes where it would be difficult to imagine the formation of ecotypes, for instance, within the ranges of species which have extensive migrations. Secondly, allopatric isolation can occur as a result of vicarious events which subdivide a species distribution by superimposing a barrier to migration somewhere within the species range or by dispersal into previously uninhabited areas. Probably the best example of the former with respect to marine fishes is the formation of the Isthmus of Panama. For the latter, the best example is the dispersal of Atlantic or Arctic Ocean fishes into the Pacific, or vice versa, when the Bering Strait opened in the late Pliocene. Thirdly, we must assume that the biogeographic relationships among the various sardine taxa have resulted from historical events in the past and are not wholly the result of present oceanographic features.

There are several predictions from population genetics theory and interpretations of biogeographic data in other species that may bear on explaining why there are so few species of sardines. Both theoretical (e.g. Templeton 1980a,b) and paleobiographic (e.g. Jablonski 1986) studies suggest that speciation

is more likely to occur in species with highly subdivided population structures, in which the average genetic distance between populations is large, than in species acting like a single panmictic population because of large amounts of gene flow. Theory suggests that genetic changes occur through natural selection and random genetic drift and that change can be much more rapid in small populations than in large populations, and these genetic changes can lead to intrinsic reproductive barriers between populations and to the formation of new species.

Genetic studies of most epipelagic marine fishes (see earlier discussion of stock structure) show that there is generally very little genetic subdivision within the range of a given species. In addition sardines have a number of behavioral characteristics which would tend to reduce their speciation rate. Sardines make extensive migrations and alter their geographical distributions in association with fluctuations in abundance or environmental perturbations. They have extended spawning seasons with multiple spawning; some spawning is observed all year in many of the warmer spawning grounds. Thus there is extensive opportunity for interbreeding between fish from different geographical areas within a current system. In addition, as previously mentioned, sardines have extremely large populations. Sardines must therefore be included in the category of species that are not likely to speciate within an individual current system. Thus we do not see sympatric or parpatric species of sardines which can be interpreted to have arisen in situ.

On a broader geographic scale there are two problems requiring explanation. One is to explain the major phylogenetic subdivision between the European sardine (*Sardina pilchardus*) and the remaining taxa in the genus *Sardinops*. The other is to explain why the populations of *Sardinops* in widely separated current systems are genetically so similar.

Before attempting to answer these two questions, it would be useful to put the biogeographic and speciation events of these taxa into a temporal context. A genetic distance can be used to estimate in a rough way the time since divergence between two related taxa (Grant 1987). Empirical calibrations of genetic distance suggest that a Nei genetic distance of 1.0 is approximately equal to 19 million yr of divergence time. However, comparisons of genetic distances between pairs of related fish taxa separated, for instance, by the Isthmus of Panama 3.1 million yr ago, show that there is a large error associated with any estimate of time. Therefore it is better to use the estimate of genetic distance along with its standard error to estimate a period of time when divergence was most likely. For the *Sardina–Sardinops* split with $D = 1.10$ (SE 0.26) this period of time extends from 16 to 26 million yr before the present; or from the end of the Oligocene to the mid-Miocene. For the various *Sardinops* splits the average D value was 0.005 (range 0.002–0.009) and the average standard error was 0.004 (range 0.002–0.008). Using the average D value and the average standard error the time of divergence of the *Sardinops* populations would have occurred from 19 000 to 171 000 BP. Both dates are in the late Pleistocene. The last glacial period is considered to have peaked about 18 000 BP and the last interglacial period was about 125 000 BP.

Given the present-day location of *Sardina pilchardus* and the apparent length of time that they have been separated from *Sardinops*, we suggest that the closure of the Tethys Sea in the late Oligocene by the colliding continental plates of Laurasia and Africa (King 1983) served as a vicariant event leading to the separation of the ancestral sardine taxa into two isolated populations. *Sardina* was then derived from the North Atlantic population and *Sardinops* from the Indo-Pacific population.

Sardina is presently restricted to the subtropical portion of the northeastern Atlantic and the Mediterranean. The absence of *Sardina* from the northwestern Atlantic, if indeed it ever existed there, could be explained by local extinction during Pliocene or Pleistocene cooling. During periods of cooling the North Atlantic was characterized by large-scale intrusions of cold polar water and during the Pleistocene much of the North Atlantic was repeatedly covered by an ice sheet (Kellogg 1980). Latitudinal isotherms were much compressed during these cooling episodes (CLIMAP 1976) and habitat alterations could have easily led to the extinction of *Sardina* in the northwestern Atlantic. The dispersal capability of *Sardina* is apparently not great enough for it to have made the upstream, westward transit across the mid-latitude Atlantic since the last glacial maxima; nor has its temperature tolerance permitted it to colonize the northwestern Atlantic by westward advection around the polar, northern rim of the North Atlantic or along the North Atlantic equatorial current.

The task of explaining the biogeographic origins and distribution of *Sardinops* is much more difficult. The minor genetic distances between populations in different current systems suggest two general hypotheses:

1) There is ongoing migration between the various current systems which keeps the populations so genetically similar.
2) *Sardinops* achieved its present distribution as the result of Pleistocene climatic changes, in which case there were probably exchanges between current systems at some time during each glacial cycle.

Before developing these hypotheses it should be noted that the earliest time that *Sardinops* could have achieved its present distribution was probably during the late Miocene. The middle Miocene fish fauna of California is quite well known and Jordan (1921) points out that, with few exceptions, the middle Miocene fauna is similar to the present subtropical fauna of California. One of his exceptions is "There are no anchovies, sardine, or true herring, the numerous herring-like forms being all of rare or extinct types." We note that the striations on the opercular bones of *Sardinops* and *Sardina* make them particularly easy to identify as fossils. It should be noted that Fitch (1969) found the otoliths of the northern anchovy, *Engraulis mordax*, and the Pacific herring, *Clupea pallasi*, but not *Sardinops* in three Pliocene deposits and numerous Pleistocene deposits. The earliest recorded occurrance of *Sardinops* in the California Current region is from Holocene sediments off Vancouver Island which were dated between 8 850 and 9 840 yr BP (Casteel 1975). The earliest recorded occurrance of *Sardinops* in the Peru Current region is not much earlier; (i.e. between 11 400 and 11 700 yr BP, DeVries and Pearcy 1982). However, we were unable to find any studies of the Miocene–Pleistocene marine fish fauna of this region.

To evaluate the first hypothesis it is necessary to evaluate the probability of exchanges of sardines between current systems under the present climatic conditions. Sardines are highly migratory, epipelagic plankton feeders and it is certainly within the realms of possibility that they could simply swim across an ocean. For example, tagged sardines have made one-way migrations between southern California and Canada (1850 km) in as little as 103 d (Jannsen 1938). On the other hand they do not occur in the northwestern or southwestern Atlantic.

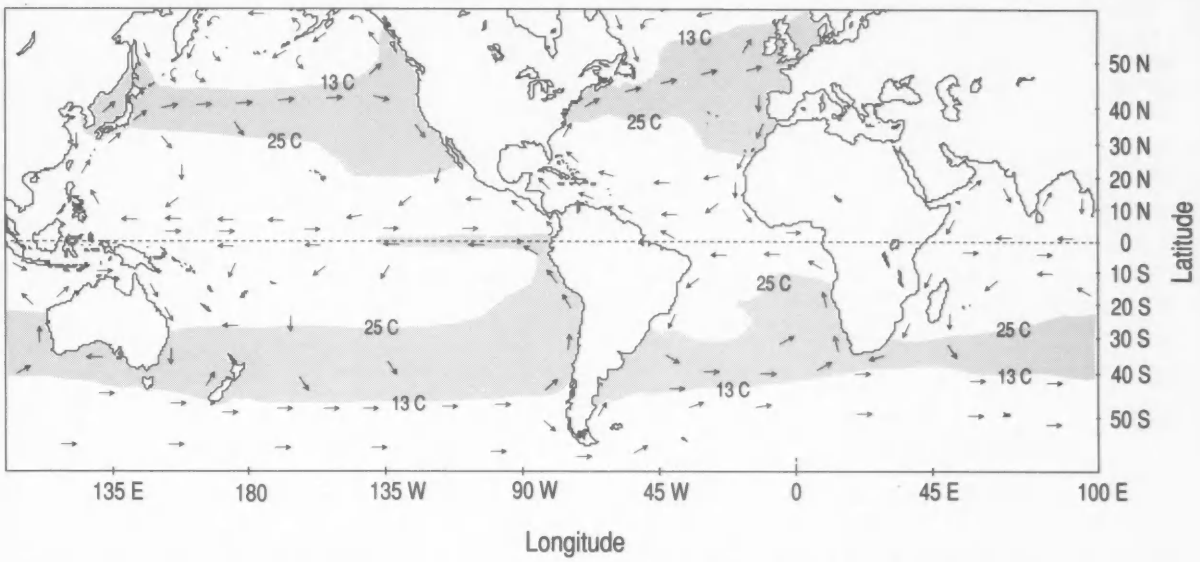

FIG. 5. Major ocean circulation features and area with summer sea surface temperatures between 13 and 25°C (shaded). Based on charts in Sverdrup et al. (1942).

The biomass levels of sardines within each of the major current systems for which population estimates exist, have at times exceeded several million tons. At these biomass levels there are undoubtedly millions of larval and juvenile sardines swept offshore and lost to the neritic populations each year. It is therefore possible that sardines could be passively advected from one current system to another.

The temperature tolerance of adult *Sardinops* is from 7 to 27°C (Tokai Regional Fisheries Research Laboratory 1960; Ahlstrom 1960) and most spawning occurs at temperatures of 13–17°C (Ahlstrom 1960). In all of the regions in which it occurs the high and low latitude edges of the normal range of *Sardinops* (Fig. 1–3) are well described by the summer 13 and 25°C isotherms (Fig. 5). This is well within the range of sea surface temperature (SST) occurring in the mid-latitudes but SST at low and high latitudes (Sverdrup et al. 1942) is near or exceeding lethal temperatures for sardines. Of course sardines could feasibly avoid the high equatorial SST by staying in the thermocline to avoid thermal stress. However, the interaction between the increased metabolic rates that would occur at higher temperatures and the lower food availability which occurs in tropical waters would be a severe limitation to sardine survival in equatorial and central water mass regions.

The most likely exchange of sardines between current systems, with the present climatic conditions, would appear to be a mid-latitude crossing from a western boundary current to an eastern boundary current via one of the west wind drift regions. Recent research on the near surface currents of the North Pacific, using drifting buoys, has shown that the average transit time across the North Pacific going east in the mid-latitudes and west in the equatorial regions is approximately the same, 700 d (McNalley et al. 1983). Sardines often live for up to 10 yr and 5-yr-old fish are quite common. It is thus feasible that there could be passive advective exchanges within an ocean basin gyral system. The major limitations to this type of exchange would appear to depend on the availability of food and encounters with predators. Sardines are essentially planktivorous species which feed on both phytoplankton and zooplankton. McGowan (1972) has described the biogeography of the major zooplankton faunal assemblages in the Pacific. He describes a "transition zone" fauna which is distributed along the warm side of the subarctic convergences associated with the west wind drift. It is obvious from a comparison of the distributions of McGowan's faunal assemblages with that of *Sardinops* that, in the Pacific, *Sardinops* populations are centered in the neritic boundaries of the transition zone fauna. This implies that sardines could remain within the transition zone fauna if, for example, they were advected eastward from the Kuroshio Current to the California Current via the North Pacific Current (i.e., the west wind drift region) but not if they were advected westward from the California Current to the Kuroshio Current via the North Pacific Equatorial Current or via the Alaska and Oyashio Currents (Fig. 5).

In this regard, Kenya (1982) has reported finding commercial quantities of sardines along a large portion of the Kuroshio extension; sardines were taken as far east as 171°E. This is approximately 2800 km from the spawning grounds of southern Honshu and approximately one third of the way across the Pacific. Kenya also found young-of-the-year sardines as far east as 166°E in July. Thus it is likely that during the Holocene there has been frequent eastward, advective mixing of sardines from the Kuroshio Current to the California Current via the west wind drift region and, by analogy, between the southern African, Australian, New Zealand, and South American populations.

Conversely westward, advective exchange via the equatorial currents would be very unlikely as it would have to occur at near lethal temperatures and within zooplankton faunas in which sardines do not normally occur. Westward, advective exchange via the Alaska Current would presently be impossible as temperatures are near, and seasonally below, the sardine's temperature tolerance range, and there is no comparable subpolar gyre

in the southern hemisphere. Westward mid-latitude exchange, via the northern and southern hemisphere west wind drift regions, would also be very unlikely as it would have to occur against the prevailing currents (Fig. 5).

Exchange of sardines between the southern and northern hemispheres would also appear to be very difficult as present sea surface temperatures near the equator are higher than those in which sardines normally occur and, for the most part, the present large scale circulation patterns would tend to hinder exchanges between the northern and southern hemispheres. If transequator exchanges are possible under the present climatic conditions they would be more likely to have occurred on the eastern edges of the oceans rather than the western edges . There are two principal reasons for this; the eastern edges of the tropical oceans are cooler than the western edges and the eastern boundary currents advect cool (sardine rich) water towards the equator whereas the western boundary currents advect warm (sardine poor) water away from the equator.

The most likely area for transequator exchanges with the present climatic conditions is the Eastern Tropical Pacific. In the eastern Pacific *Sardinops* occurs to southern Baja California (24°N) in the California Current and to the equator in both Ecuador and the Galapagos Islands. *Sardinops* does not presently occur in the Eastern Tropical Pacific; however, it can at times survive there as fishing vessels have successfully transported it from the Galapagos Islands to the Central American tuna fishing grounds as live bait (Alverson and Shimada 1957). Present SST in the Eastern Tropical Pacific (Sverdrup et al. 1942) is near the lethal limit for sardines; however, the prevailing nearshore currents would favor dispersal from the Peru Current to as far north as southern Mexico. Seasonally, currents are favorable as far as 28°N in the Gulf of California (Baumgartner and Christensen 1985). Conversely southern dispersal from the California Current to the Peru Current would be hindered by the prevailing currents.

The second area in which transequator exchanges might occur with the present climatic conditions is in the Guinea Current region. *Sardina* presently occurs as far south as 15°N in the Canary Current and *Sardinops* occurs as far north as 16°S in the Benguela Current. Sea surface temperatures over most of the Guinea Current region (Sverdrup et al. 1942) are near or above the lethal limits for sardines. The region has somewhat lower SST in August with SST over some of the area below 25°C: however, at this season SST in the area between the Canary and Guinea currents is above 27°C. The prevailing current in the Benguela and Canary Currents is equator-ward. Sardines could therefore possibly be advected to the equator; however, once across it they would have 15 degrees of latitude to cross, against the prevailing currents at near-lethal SST, before reaching sardine habitat in the opposite hemisphere. Thus transequator exchanges in the Atlantic appear to be impossible for either *Sardina* or *Sardinops*.

The second hypothesis is supported by the fact that the apparent time of divergence between populations in the different current systems is placed in the Pleistocene, a time of both global oceanic cooling and warming that would have enhanced migration from one current system to the other. We note that the genetic distance of 0.003 between the Japanese and Californian sardine populations is on the same order of magnitude or less than genetic distances observed between eastern and western Pacific ocean populations of Pacific herring, *Clupea pallasi* (Grant and Utter 1984), and Pacific cod, *Gadus macrocephalus* (Grant et al. 1987).

Eastward exchange between western and eastern boundary currents during the Pleistocene would have been possible with the same explanations as those given above for the present climatic conditions. It is likely, however, that conditions are more favorable at some stages in the glacial cycle than at others. Westward exchange during glacial maxima via the equatorial currents would have been nearly as unlikely as at present as there is little temperature difference in the tropics between glacial maxima and minima (CLIMAP 1976). However, there is considerable difference between temperatures at glacial maxima and minima in the subpolar regions (CLIMAP 1976) and paleosediment analyses show that the North Pacific subpolar gyre is an area with maximum faunal changes between glacial maxima and minima (Ruddiman 1985). Westward exchange may, therefore, have been possible in the North Pacific via the Alaska Current when conditions were warmer than at present (i.e., in the Pliocene or during extreme interglacial periods during the Pleistocene).

The evidence discussed earlier suggests that with the present climatic conditions transequator exchanges of sardines are not possible anywhere except in the Eastern Tropical Pacific, and they would have to be considered very unlikely even there. However, recent evidence from oxygen isotope and radiolarian analyses of ocean cores suggests that at the last glacial maximum (18 000 yr BP) the August 25°C isotherms were displaced southwards to southern Mexico (18°N) and northwards to southern Costa Rica (8°N) and maximum mean SST in the eastern tropical Pacific was only 26°C (Ruddiman 1985). Jones and Hassen (1985) suggest that contact between the Tethys-derived coral fauna in the Caribbean and Tropical Pacific was lost in the late Pliocene, following development of the Panama land bridge, and the virtual extinction of the eastern Pacific coral reef fauna ensued during Pleistocene glaciations. There is, therefore, considerable evidence that implies that climatic conditions in the Eastern Tropical Pacific would have been cool enough to allow *Sardinops* from the Peru Current to cross to the California Current during Pleistocene glacial maxima.

In summary we suggest:

1) Eastward, advective exchange of *Sardinops* between western and eastern boundary current systems via the west wind drift regions and within the transition zooplankton faunal zones is possible with the present climatic conditions.
2) Westward, advective exchange of *Sardinops* from the California Current to the Kuroshio Current via the subpolar gyre is not presently possible, but it would have been possible during the late Miocene and early to mid-Pliocene when sea surface temperatures were warmer than those that have existed since the onset of northern glaciation in the late Pliocene. It may also have been possible during extreme interglacial maxima.
3) Westward, advective exchange is and was not possible via the equatorial currents due to their high SST and different zooplankton fauna.
4) Westward exchange, against the prevailing current, is and was not possible within the west wind drift regions.
5) Trans-equator exchange is only possible in the eastern Pacific and is most likely to occur during glacial maxima.

If the above statements are accepted, then it can be postulated that the present distribution of *Sardinops* was derived from the Indo-Pacific segment of the original Tethys sardine population. *Sardinops* is most likely to have achieved its present distribution by first dispersing from the southern Indian Ocean to

Australia, New Zealand, and Pacific South America via the southern hemisphere west wind drift. The absence of *Sardinops* in the southwestern Atlantic Ocean can be explained by the fact that *Sardinops* from the Benguela Current would have to move against the west wind drift current to colonize the region and *Sardinops* from the Pacific could not reach the region as the southern tip of South America extends into water that is too cold for *Sardinops*.

The present southern hemisphere distribution was probably achieved by the late Miocene or early Pliocene, a period when ocean temperatures were decreasing and oceanic productivity was increasing. In the eastern Atlantic, paleo-oceanographic reconstructions show that, although there was considerable cooling in temperate areas, Pleistocene equatorial waters remained nearly as warm as present day equatorial waters (Thunell and Belyea 1982). Indeed, there is no evidence to show that *Sardinops* (or *Sardina*) crossed the equator in this region. The colonization of the North Pacific apparently did not occur until after the closing of the Isthmus of Panama and the onset of extensive northern hemisphere glaciation in the late Pliocene; it is most likely to have occurred in the Pleistocene when the minimum ocean temperatures associated with a glacial maxima would have resulted in climatic conditions which would most favor the dispersal of *Sardinops* from the Peru Current to the California Current. There is a possibility that *Sardinops* achieved its present distribution by reaching the Kuroshio Current, via the North Pacific subpolar gyre, in the late Pliocene; however, this probably occurred during an extreme interglacial period in the Pleistocene.

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LETTER AND COMMENT/ LETTRE ET COMMENTAIRE

Comment on Functional Maturity in Small Male Snow Crab (*Chinonoecetes opilio*): Sizing Up the Evidence

In a recent paper published in this journal, Ennis et al. (1988) examined snow crab collected during the annual spring breeding migration to shallow water in Bonne Bay, Newfoundland, from 1983 to 1987. The principal information consisted of the sizes of crabs in sexual pairs and the presence of old and new spermatophores in the spermathecae of some females. In addition, the authors made observations on copulatory behavior and subsequent egg extrusion of crabs held in tanks. From this work, Ennis et al. (1988) argued that males smaller than the minimum legal size (95 mm carapace width, CW) ". . . can replace large males in breeding activity in a snow crab population" and further concluded that "population reproductive potential is maintained at a high level despite high exploitation rates on males ≥ 95 mm." We believe that both these statements are misleading because of shortcomings which we have grouped into the following categories: (1) ambiguous observations, (2) inadequate data, (3) circumvented issues of central importance, (4) untested assumptions which may affect the conclusions, and (5) unfounded general conclusion. We shall address each of these in turn.

1. Ambiguous Observations

The authors state that they determined the presence of old and new spermatophores in the spermathecae of the females examined, based on distinguishing features given by Adams and Paul (1983), Taylor et al. (1985), and Paul (1984). Inspection of these papers reveals either no reference to spermatophore features (Adams and Paul 1983), or else a cursory description based on visual inspection of old and new ejaculate, which consists of matrix and spermatophores (Paul 1984; Taylor et al. 1985). Assuming that the authors were indeed referring to ejaculate rather than spermatophores, the distinction between "old" and "new" may itself be uncertain. In a recent paper on the functional anatomy of snow crab reproductive systems, Beninger et al. (1988) presented the hypothesis that spermathecal contents may be rehydrated either during copulation or during fertilization in the absence of a male. In such cases, it would be impossible to ascertain that "old" ejaculate is yellowish and waxy, while "new" ejaculate is whitish and fluid. In addition, the assumption that ejaculate always contains spermatophores may itself be unfounded. In their study, Beninger et al. (1988) reported that of twelve females with distended spermathecae, only one contained spermatophores within the matrix.

A further ambiguity concerns the characterization of multiparous versus primiparous females. Ennis et al. (1988) considered that, on the basis of darkened grasping marks, all their females were multiparous (i.e. females that had extruded a second or subsequent batch of eggs). Again, for distinguishing characteristics of fresh and old marks, the authors cited a paper (Paul 1984) which on inspection does not provide such information, and, moreover, deals with a congener of *C. opilio*. Regardless, the relative "age" of grasping marks alone is not necessarily a reliable indicator of reproductive history. Primiparous females (females that have mated at their terminal molt to maturity and extruded a single egg batch) may, towards the end of their brooding period, exhibit "old" marks from their primiparous mating and be misidentified as multiparous. Similarly, primiparous females that were unscathed by mating could conceivably extrude a second egg batch utilizing stored sperm, thus avoiding grasping marks again, and be erroneously classed primiparous. Although these are subtle points, we believe that consideration of the precise reproductive history of paired females, when taken in tandem with the size and morphometric maturity status of their partners, is essential to elucidating reproductive processes in *C. opilio*.

2. Inadequate Data

Close scrutiny of the arguments in Ennis et al. (1988) for a trend in the numbers of males < 95 mm CW participating in the breeding migration reveal that they are based on scant data. Back-calculation from the percentages and totals provided gives the following actual numbers of paired males < 95 mm CW sampled:

1983 — 1 out of 67
1984 — 3–6 out of 304
1985 — 2–4 out of 199
1986 — 1 out of 15
1987 — 10 out of 31

Overall, Ennis et al. (1988) is based on a trend for 17–22 out of 616 male snow crabs collected over 5 yr, with approximately half of the "small" males being sampled in a "surge" during the final year. Intuitively, such sample sizes are unreasonably small given the far-reaching conclusions of their study. Further, on the same evidence, the authors contend that the increased incidence of "small" males participating in the breeding migration after 1986 appears to be ". . . a direct result of reduced abundance of commercial-size males caused by the rapid development of an illegal fishery and, as a consequence, less competition between males for possession of females" (p. 2108). Notwithstanding the obvious problem of sample size, these observations may also be explained by a recruitment pulse of small males coming through the system, perhaps as a consequence of exploitation (see Waiwood and Elner 1982). Clearly, additional factors, such as shifts in historical size-frequency profiles, shell condition and relative abundance-at-size indices, need to be considered if we are to resolve whether there was an increase in the number of pairs including small males as a consequence of an insufficient number of large males or because there were relatively more smaller males (or a combination of both scenarios).

3. Circumvented Issues

Although the paper by Ennis et al. (1988) is entitled "Functional maturity in small male snow crabs . . .," the exact sig-

nificance of the term "functional maturity" is not given; indeed, it is also lacking in the literature. Conan and Comeau (1986) define functional maturity as the "aptitude to mate successfully." This definition is unsatisfactory, as it suffers from the ambiguity of the term "mate."

The tone of Ennis et al. (1988) would suggest that functional maturity is the ability to copulate and transmit ejaculate, as a result of which eggs are extruded. Since it is unknown whether the spermatozoa of small crabs are in fact viable, and it is equally unknown whether the extruded eggs are fertilized, the "functional maturity" observed here does not necessarily mean successful fertilization. Surely this must be the acid test of true functional maturity and it should be demonstrated if the term is to be used — especially if the authors then equate eggs with recruits (p. 2109). Mating experiments between newly-pubescent females and "small" males, followed by developmental observations on the egg clutches, would suffice to resolve this important question.

Another central question which is circumvented in the paper by Ennis et al. (1988) is that of morphometric maturity (see O'Halloran 1986). The Bonne Bay site offers a unique opportunity to definitively test the controversial hypotheses proposed by Conan and Comeau (1986), specifically:

— Only morphometrically mature males, in terminal molt status, ⩾ 95 mm CW are functionally mature.

— Morphometrically immature, non-terminal molt males up to 120 mm CW are not functionally mature.

Unfortunately, Ennis et al. (1988) either failed to distinguish, or chose not to provide, the morphometric maturity status of their paired males; thus, they cannot fully interpret their findings and are premature in extending their arguments to the Atlantic snow crab fishery as a whole. Further, we believe that by evading the issues of male terminal molt and maturity morphometrics, an important opportunity to advance snow crab biology has been missed while at the same time a potentially misleading research direction may have been established. Without the morphometric measurements Ennis et al. (1988) adds little new knowledge; the fact that "small" males per se can mate is not novel, as Watson (1972) observed males as small as 60 mm CW in primiparous pairings with larger females in the laboratory. Also, Taylor et al. (1985) and Hooper (1986) reported males of 89 mm CW and 93.8 mm CW, respectively, paired with multiparous females in Bonne Bay, Newfoundland. The critical issue is the size (CW) of the paired males *plus* their morphometric maturity or immaturity status. Also of interest is the primiparous or multiparous status of the females paired with each of the four male categories (i.e., 1 — small morphometrically immature, 2 — small morphometrically mature, 3 — large morphometrically immature, and 4 — large morphometrically mature). Clearly it still remains to be tested whether small (or even large) morphometrically immature males can sustain reproductive output as well as small or large morphometrically mature males. The relative number of males in each of the categories is seen to shift over time (Conan and Comeau 1986). Conceivably then, removal of large males in one stock could have profoundly different consequences for reprodutive output as compared to another stock, depending on the relative numbers of small morphometrically immature and morphometrically mature males in each stock and their ability to mate with primiparous and/or multiparous females (quite irrespective of the unaddressed problem of the relative contributions of primiparous versus multiparous females to total egg production).

4. Untested Assumptions Which May Affect Conclusions

The paper by Ennis et al. (1988) contains (either explicitly or implicitly) several untested assumptions which, if incorrect, may greatly affect their conclusions. Among these, the following are noted:

i) The authors "conclude that sublegal-size males can substitute for larger commercial-size males in normal mating activity in a *C. opilio* population" (p. 2109). This conclusion is based on two unproven assumptions:

   a) that the number of spermatozoa transmitted by "sublegal" males is equivalent to that transmitted by commercial-size males, or else that the ratio of spermatozoa to eggs is sufficient to fertilize all eggs regardless of male size. Conan and Comeau (1986) state that the vasa deferentia of small (<95 mm CW), *morphometrically mature* snow crabs in heavily-exploited populations contained more spermatophores than those of the commercial-size crabs, which were presumably captured before being able to form a larger number of spermatophores. Although no quantitative data were presented, such an observation should serve as a warning against comparing the spermatophore count of sublegal morphometrically mature males to that of commercial-size males which are so heavily exploited that most do not form a full complement of spermatophores. In addition, it is not known whether spermatophore size varies with crab size, or, more precisely, whether the mean number of spermatozoa within the spermatophores is related to crab size. The observations of Conan and Comeau (1988) also serve to underscore once again the importance of morphometric status in evaluating reproductive potential.

   b) that the spermatophores and spermatozoa of sublegal males function as well as those of commercial-size males.

ii) It is at present unknown what the exact fertilization events in snow crab are, as they take place within the "black box" of the spermatheca. Given that "old" spermatophores from a previous copulation with a "large" male may be present in some females which were observed to be paired with small males, it is impossible to conclude that the extruded eggs were fertilized by the small males (except in the case of previously virgin, primiparous females, which cannot be distinguished with certainty — see section 3 above). Indeed, given the lack of any direct observations of copulation, it is impossible to conclude that the small males actually copulated with these females, and equally impossible to conclude that ejaculate was transmitted (assuming such ejaculate to contain viable sperm).

iii) In order for the above-cited conclusion to be tenable, the number of eggs extruded by a female which had copulated with a small male (supposing all of the above assumptions to be true) would have to be equal to that extruded by a female which had copulated with a commercial-size male. As the authors present no data on egg counts, this assumption simply cannot be made.

5. Unfounded General Conclusion

The general conclusion presented by the authors has far-reaching implications for fisheries management of snow crab

in the Atlantic region, and, by extension, for the socio-economic situation of those involved in the fishery:

> "From the available evidence, we conclude that production of viable eggs, even in heavily-fished *C. opilio* stocks, is maintained to the right of the ascending limb of a stock-recruitment curve whatever its general shape might be for this species" (p. 2109).

This conclusion cannot be derived from the results presented, as the authors only demonstrate that "small" males can participate in some reproductive activities. This may or may not have implications for the production of fertilized eggs, depending on which, if any, of the previously-mentioned assumptions are in fact valid.

It is disturbing that the authors arrive at a conclusion concerning the hypothetical production of viable eggs when females pair with small males, even though they present no data on the actual viability of the eggs extruded by such females. Another leap of faith is taken by implicitly equating eggs with recruits. As the relationship between the two is never equal and often not proportional (Cobb and Caddy 1989) this statement should be regarded as conjectural until proven otherwise. A more judicious choice of words might have been "stock-egg curve," although here again all of the previously-mentioned assumptions would have to be proven valid in order to reach such a conclusion.

Conclusions

It is clear that the concluding statements by Ennis et al. (1988) are, at best, hypotheses requiring considerable careful research in order to be verified. In addition, the numerous assumptions upon which these hypoptheses are based should themselves be the objects of detailed and duly-published studies. The *C. opilio* fishery is characterized by effort directed exclusively at larger ($\geq$ 95 mm CW) males; most females reach their final size well below this limit. It is therefore of special importance to understand reproduction in this species, and in particular to assess the effect of selective removal of large males (Elner et al. 1986). Given the now-evident complexity of reproduction in *C. opilio*, research in this field should proceed cautiously and meticulously. Unfortunately, biological understanding of snow crab has been hindered by a series of uncritically accepted "truths" based on conventional wisdom rather than experimental testing, which are only recently being confronted and rejected (see Bailey and Elner 1989 for review). The situation with snow crab is possibly no less poignant than for other commercial invertebrates but has probably been exacerbated by pressures on biologists to provide rapid insights for managers of this particularly volatile fishery. Our challenge of Ennis et al. (1988) is primarily intended to help avoid the further acceptance of "truths" until critical scientific testing has been performed, rather than to argue that any particular facet of their work is necessarily, or will ultimately be proved, incorrect — Robert W. Elner, *Department of Fisheries and Oceans, P.O. Box 550, Halifax, N.S. B3J 2S7,* and Peter G. Beninger, *Département de biologie et Centre de recherche en sciences de l'environnement, Université de Moncton, Moncton, N.B. E1A 3E9.* (JA064).

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Reply to Comment by R. W. Elner and P. G. Beninger

Male snow crabs achieve sexual maturity over a size range well below the 95 mm CW size limit applied in managing the fishery in Atlantic Canada. However, Conan and Comeau (1986) stated that there is no evidence that sublegal (i.e. <95 mm CW) males can mate in nature with multiparous females. The validity of this statement is of considerable importance to snow crab fishery management.

In our sampling of sexually-paired snow crabs obtained in Bonne Bay from 1983 to 1986, 1.2% of the males were <95 mm CW and the smallest was 76 mm. In 1987 sampling, however, this percentage increased to 32.3% and the smallest was 62 mm. All females in these samples were multiparous. We reported these observations in Ennis et al. (1988a) primarily because they were evidence that sublegal males can mate in nature with multiparous females. Observations on the spermathecal contents of the diver-caught, paired females which had been paired with males <95 mm and with males $\leq$95 mm along with similar observations on male/female pairs selected from trapped samples and held in cages and tanks were also presented as evidence that males <95 mm can mate with multiparous females. We concluded that sublegal-size males can substitute for larger commercial-size males in normal mating activity in a *C. opilio* population and that in the male-only snow crab fishery in Atlantic Canada with a minimum legal size of 95 mm CW, population reproductive potential is maintained at

a high level despite high exploitation rates on males ≥95 mm. Elner and Beninger believe that both of these conclusions are misleading because of shortcomings which they grouped under five headings which we also use to organize our reply.

1. Ambiguous Observations

On the basis of the presence of spermatophores in the vasa deferentia, Watson (1970) determined that male *C. opilio* in the Gulf of St. Lawrence mature over the 51 to 72 mm CW size range. Based on sampling in the Gulf of St. Lawrence as well, Conan and Comeau (1986) found that all males >60 mm bore spermatophores in their vasa deferentia. Watson (1970) found spermatophores in the spermathecae of all of 25 mature females with new, hard shells and in 37 of 46 mature females with old, hard shells. He suggested that those without spermatophores had spawned several times since reaching maturity (i.e. mated at their molt to maturity but not subsequently). Elner and Gass (1984) found in a sample of 98 mature females taken off northwest Cape Breton Island (Gulf of St. Lawrence) in November 1983 that 98% had sperm in their spermathecae.

In the laboratory, Adams and Paul (1983) mated females of the closely related species *C. bairdi* that had recently undergone or were about to undergo their maturity molt with males ranging in size from 65 to 140 mm CW (24 specimens from 65 to 95 mm). All females received enough sperm to fertilize their first clutch of eggs (i.e. primiparous) which was extruded shortly after mating and 93% had enough sperm remaining in their spermathecae following egg extrusion to fertilize additional egg clutches. Paul (1984) held multiparous *C. bairdi* at ratios up to one male to 13 females. At sex ratios of one male to eight or fewer females, 96 to 100% of the ripe multiparous females mated (i.e. fresh sperm in their spermathecae) before extruding eggs. At sex ratios greater than one to eight, an average of 74% of the females mated. In a sample of 64 multiparous *C. bairdi* collected in May, 97% had freshly deposited sperm in their spermathecae (Paul 1984).

Paul (1984) described fresh ejaculate in the spermathecae of female *C. bairdi* as a dense, white semisolid and ejaculate that had been stored from previous matings as a distinctive amalgamated mass slightly yellow or brown in color. This description followed a laboratory study (Adams and Paul 1983) which involved the detailed examination of spermathecae of primiparous females shortly after they molted to maturity, mated and extruded their first clutch of eggs.

When we began detailed sampling of sexually-paired *C. opilio* in Bonne Bay in 1984, we found in the spermathecae of some females what we could readily distinguish as fresh and old ejaculate as described by Paul (1984) for *C. bairdi* females. In a sample taken between April 24–27, 1984 (near the beginning of the breeding migration to shallow water), 48.6% of 35 paired females had new and old ejaculate (Taylor et al. 1985). Six spermathecae with old ejaculate only and six with both new and old from this sample were prepared histologically for detailed examination. Spermatophores were clearly visible in all 12 specimens and in sections from spermathecae containing old and new ejaculate it was very clear that spermatophores were much denser than in sections from spermathecae containing only old ejaculate. In another sample of 42 paired females taken one month later, 92.9% had new and old ejaculate.

Elner and Beninger correctly assume that what we reported in Ennis et al. (1988a) were observations on ejaculate rather than on spermatophores per se and suggest that the assumption that ejaculate always contains spermatophores may be unfounded. The foregoing, however, demonstrates that in ejaculate distinguishable as new, spermatophores are always present and in old ejaculate spermatophores are present in the majority of cases.

Elner and Beninger cite Beninger et al. (1988) who found that only one of 16 females they examined had spermatophores in its spermathecae and contrast this with the results of Elner and Gass (1984) (cited earlier). The observation also contrasts with results of others we have cited. Beninger et al. (1988) also considered enigmatic their observation that all but one of the 12 females they examined histologically had distended spermathecae without spermatophores. Their sample of ovigerous females came from an area off eastern Cape Breton Island (Area 5) where the fishery had collapsed from landings of 680 t in 1979, just a couple of years after it started, to 45 t in 1984. Landings dropped further to 29 t in 1985 but have since recovered somewhat to 174 t in 1988 (Elner et al. 1989). The fishery reduced the population to a very low level of abundance over a short period of time and there was very little recruitment to replace the fishery removals (Elner and Robichaud 1985).

In July–August samples of mature females from this population, the percentage ovigerous was around 100% from 1978 to 1982 (Elner and Robichaud 1986). In 1983 it dropped to 95% in one sample and to 90% in another. In a September 1984 sample the percentage had dropped to 77% and a large proportion of these appeared to have reduced clutch sizes (Elner and Robichaud 1985). Elner and Robichaud (1985) state "most of the females (i.e. September 1984 sample) had heavily fouled and aged carapaces; many had missing appendages" and "overall evidence suggests that the long-term lack of recruitment to Area 5 is resulting in stock senescence and extinction: males of commercial size are rapidly being extinguished and females are becoming either too old to reproduce or are failing to copulate". Clearly, the comments by Elner and Beninger based on the sample of ovigerous females taken in this area in August 1984, held in captivity and sacrificed in November 1984, are biased and suspect.

Beninger et al. (1988) attribute the extremely viscous consistency of the spermathecal matrix to water loss during storage. They also postulate that the highly viscous ejaculate is diluted with sea water during copulation or immediately prior to later egg extrusion and that stored ejaculate from a previous mating is rehydrated by entry of sea water into the spermathecae at the same time. Elner and Beninger suggest that in such cases it would be impossible to ascertain that "old" ejaculate is yellowish and waxy, while "new" ejaculate is whitish and fluid. Assuming that rehydration of old ejaculate does occur during copulation there is no indication in Beninger et al. (1988) or any explanation in the comment by Elner and Beninger why old and new ejaculate would be impossible to distinguish in females that had recently mated. Elner and Beninger also offer no suggestion regarding what Ennis et al. (1988a) refer to as old and new spermatophores (i.e. ejaculate) in the spermathecae of sexually-paired females taken during the breeding season might be.

We have examined spermathecae of laboratory-held primiparous females shortly after mating. These contained ejaculate identical in appearance to what we referred to in Ennis et al. (1988a) as new spermatophores, which we are confident was ejaculate from a very recent mating which contained new spermatophores. Our statistical analyses of the data showed no significant differences in the percentages of females with new eja-

culate in their spermathecae for those paired with males <95 mm and those with males ≥95 mm. These along with the incidence of males <95 mm participating in the breeding migration were the key observations reported in Ennis et al. (1988a).

Elner and Beninger also consider our characterization of multiparous versus primiparous females ambiguous. In Materials and Methods we mentioned noting the presence of old and new grasping marks on the legs of diver-caught, paired females and that the old marks of a previous mating are darkened and readily distinguished from the fresh marks of a recent mating. Paul (1984) was cited in connection with these observations. Admittedly, Paul's (1984) observations were on *C. bairdi* and he did not provide a description per se of old and new grasping marks. However, he did mention observations on fresh grasping marks left by a recent mating in several places in his paper. It is not difficult to glean from Paul (1984) that old and new grasping marks are readily distinguishable especially after seeing both on many individual sexually-paired females. Elner and Beninger incorrectly stated that we identified all our females as multiparous on the basis of darkened grasping marks. Our identification of females as multiparous was based primarily on shell condition which, as mentioned in our Materials and Methods, was also recorded. We agree with Elner and Beninger that relative age of grasping marks alone is not necessarily a reliable basis for identification of primiparous and multiparous females.

All but 11 of 549 paired females collected in 1984 to 1987 were old, hard-shell animals which, in our view, were unquestionably multiparous. The 11 had marginally newer shells and were categorized as new, hard-shell animals. Observations on recaptured crabs tagged in soft-shell condition (Taylor, unpublished data) indicate that these most likely molted up to two years previously. We feel confident in classifying these 11 as multiparous also.

2. Inadequate Data

In their summary, Elner and Beninger misrepresent our data on the numbers of sexually-paired males <95 mm collected in Bonne Bay from 1983 to 1987. Our statistical analyses demonstrated that the increase in percentage of males <95 mm participating in the annual breeding migration from an average of 1.2% (7 out of 585) for 1983 to 1986 to 32.3% (10 out of 31) in 1987 was significant.

We cited Ennis et al. (1988b) in which catch rates of males ≥95 mm in research trap fishing in deep water in Bonne Bay dropped very dramatically between 1985 and 1987 as evidence that the abundance of large males in the population had been substantially reduced. There has been no legal snow crab fishery in Bonne Bay and our evidence that an illegal fishery developed is anecdotal. Actual data from an illegal fishery are difficult to obtain but from extensive contact with Bonne Bay residents there is no question that poaching of snow crabs became quite prevalent during the period in question. The Bonne Bay snow crab population occupies a relatively small area and it is quite feasible that poaching could have a significant impact. Also, since 1983 research sampling of snow crabs in Bonne Bay has been extensive and may have contributed to the reduced abundance of large males in the population.

We contended that the increased incidence of males <95 mm participating in the breeding migration in 1987 was a direct result of reduced abundance of commercial-size males caused by a rapid development of an illegal fishery and, as a consequence, less competition between males for possession of females. This was based on all of the foregoing evidence and not, as Elner and Beninger state, on the incidence of males <95 mm participating in the breeding migration alone.

At a CAFSAC meeting in January 1989 we presented a paper (Ennis et al. 1989) describing our sampling in Bonne Bay during the 1988 breeding migration. In 1988 males <95 mm increased significantly from 32.3% in 1987 to 87.5%. For the first time there was also a significant decrease in the mean size of females participating and new-shell animals increased from 12.9% to 80.0% for males and from 0% to 79.3% for females between 1987 and 1988. We also provided a detailed summary of data from our research fishing and suggested that the shift from a population dominated by large, old-shell individuals in 1984 to one dominated by smaller, new-shell animals in 1988 was related to a rapid decline in the virgin population and a coincident strong pulse of recruitment.

Catch rates indicate that abundance of mature females also declined rapidly between 1985 and 1987. Anecdotal information indicates that gillnets were used extensively in illegal crab fishing in Bonne Bay. These catch females and even though they are unattractive for harvest because of their small size and negligible meat yield, they would likely be destroyed because of the difficulty of removing them intact for release.

Although illegal fishing seems to be the most plausible explanation, natural mortality due to senility may also have contributed to the population decline. The small number of pairs collected in 1986 and 1987 reflect the reduced abundance. Of the 10 paired males <95 mm collected in 1987, six were old shell, and all females, including those paired with larger males, were old shell. The four new-shell males ranged from 62 to 79 mm CW and may have been part of the recruitment pulse which predominated in 1988 when the total number of pairs collected increased to 208.

The increased participation in the breeding migration by males <95 mm is related to declining abundance of large males in the population as well as to increased abundance of small males as part of the recruitment pulse. This is evident in the details provided in Ennis et al. (1989) of which Elner was fully aware before his comment with Beninger was submitted. Whether less competition between large and small males for possession of the available females results from reduced abundance of large or increased abundance of small animals is immaterial in the context of Ennis et al. (1988a). The important thing our observations demonstrate is that small males will participate in breeding activity in a snow crab population when competition from large males is reduced.

Butler (1960) also found evidence of extensive mating activity by sublegal males in an exploited *Cancer magister* population but suggested that in an unfished population their breeding activity would be restricted by competition with legal-size males.

3. Circumvented Issues

Watson (1970) found many spermatophores in the vasa deferentia of a male snow crab as small as 51 mm CW and in this specimen the vasa deferentia filled most of the body cavity. That small males produce substantial quantities of sperm is quite clear.

In Ennis et al. (1988a) we did not provide a definition per se of functional maturity. The focus of the paper, however, was obviously the ability of males <95 mm to mate successfully (i.e. deposit sperm bearing ejaculate in spermathecae) with

multiparous females. We believe that this ability was demonstrated but we agree with Elner and Beninger that knowing whether sperm from small males (i.e. <95 mm CW) are viable is important.

Watson (1970) described a mating in the laboratory between a newly-molted female and an 83 mm CW, hard-shell male. Shortly after mating the female extruded a clutch of eggs which proved to be fertile. Taylor et al. (1985) reported a 1.4% incidence of dead eggs on sexually-paired females collected in Bonne Bay in 1984. In 1988, following the increased incidence of males <95 mm in the 1987 breeding migration, none of 79 paired females which had not yet finished liberating larvae had dead eggs and among females caught in traps during May-June none of 20 with eyed eggs and only 1 of 118 with orange eggs carried any dead eggs.

The study by Adams and Paul (1983) on *C. bairdi* leaves no doubt regarding the viability of sperm from small males in that species. They mated primiparous females with males ranging from 65 to 140 mm CW. All 62 females, including 24 which mated with males 65–95 mm, produced full egg clutches which were fertilized and 93% had enough sperm remaining in their spermathecae to fertilize additional egg clutches. They found no clear relationship between male size and sperm remaining in spermathecae after extrusion. Also, males ≤95 mm mated successfully with several primiparous females all of which extruded fertilized egg clutches shortly after mating. Adams and Paul (1983) further demonstrated that the number of fertilized eggs extruded was not related to male parent size. All 18 females which were mated with males smaller than themselves by as much as 35 mm CW produced fertilized eggs and they found no significant difference in the number of fertilized eggs extruded by these and 12 other females mated with males larger by at least 25 mm.

Laboratory studies on reproduction in *C. opilio* have been very limited. They do show, however, that, similar to *C. bairdi*, males can mate with several females over a short period of time and females can fertilize at least one egg clutch using sperm stored from a previous mating (Watson 1972). There is no reason to suspect that the preceding observations on *C. bairdi* would not also apply to *C. opilio*.

Direct evidence that *C. opilio* males <95 mm CW produce viable sperm is limited to the observation by Watson (1970) cited earlier. Elner and Beninger provide no reason to suspect that the sperm produced by small males would not be viable. We consider this a non-issue and their suggestion that we have circumvented it is unfounded.

We disagree with Elner and Beninger that the issue of morphometric maturity in male snow crabs is a central question which was circumvented in Ennis et al. (1988a). In the context of our paper, morphometric maturity was not a consideration. This issue is receiving considerable attention in current research on snow crab in Atlantic Canada but its implications for crab fishery management, especially with respect to maintaining population reproductive potential at a high level, have yet to be determined.

In Ennis et al. (1988b), data on morphometric maturity which we had at the time were presented. This included one 99 mm male out of 31 sexually-paired males collected in Bonne Bay in 1987 and three ranging from 62 to 72 mm which mated successfully with multiparous females in the laboratory that were considered to be morphometrically immature. Further data were presented in Ennis et al. (1989) based on sampling in Bonne Bay in 1988. Among 170 sexually-paired males <89 mm, none was morphometrically immature. At sizes ≥89 mm, 4 out of 33 were categorized as immature. In a trap-caught sample from deep water taken over the same period, morphometrically immature specimens were prevalent over the 62 to 90 mm size range but absent at larger sizes. These observations suggest that at sizes <89 mm morphometrically immature males may be excluded from participating in breeding activity in the wild but at larger sizes this apears not to be the case.

We did not consider morphometric maturity in Ennis et al. (1988a) but clearly, we have not circumvented the issue.

4. Untested Assumptions Which May Affect Conclusions

In the first part of their comment under this heading, Elner and Beninger state that our conclusion that sublegal-size males can substitute for larger commercial-size males in normal mating activity in a *C. opilio* population is based on two unproven assumptions: one that the number of sperm transmitted by sublegal males is equivalent to that transmitted by commercial-size males or else that the ratio of sperm to eggs is sufficient to fertilize all eggs regardless of male size, and two that the spermatophores and sperm of sublegal males function as well as those of commercial-size males.

Regarding the first of these assumptions, their reference to results of Conan and Comeau (1986) is confusing in terms of relevance here. Conan and Comeau (1986) state in their results, but without providing the actual data, that the smaller morphometrically mature males bore more spermatophores in their vasa deferentia than the larger ones, except in Bonne Bay. They suggest that in heavily exploited stocks, such as the southwest Gulf of St. Lawrence where their samples were taken in summer 1984 (except for a diver-caught sample of mating pairs from Bonne Bay in May 1984), the proportion of males caught as soon as they reach morphometric maturity becomes very high and little time is available for these to accumulate spermatophores. But Conan and Comeau (1986) also stated in their results that all males larger than 60 mm bore spermatophores independent of their morphometric state of maturity. Their observation of few spermatophores in large males from the southwest Gulf might also be explained by the possibility that these had mated several times recently.

In Ennis et al. (1988a) we made no assumption that the number of sperm transmitted by sublegal and commercial-size males were equivalent, but we did assume that a multiparous female that had recently mated with a sublegal male would have sufficient sperm to fertilize whatever number of eggs it extruded. Our citations in the preceding section support this assumption as well as the second assumption considered here regarding spermatophores and sperm of sublegal males functioning as well as those of commercial-size males.

In part two under this heading, Elner and Beninger raise the questions: were eggs fertilized by new sperm from a recent mating with a small male or by old, stored sperm from a previous mating with a large male, and did small males actually copulate with the females with which they were paired. We stated in Ennis et al. (1988a) that it would not have been possible to determine whether newly-extruded eggs had been fertilized by sperm from the recent or a previous mating. In the absence of mating, female *C. opilio* can fertilize at least one and female *C. bairdi* up to two batches of eggs using stored sperm. However, when females mate, egg extrusion occurs very soon afterwards and it seems likely that the eggs would be fertilized by sperm from the recent mating. Observations by Ben-

inger et al. (1988) support this. We also stated in Ennis et al. (1988a) that we could not be certain that the new spermatophores (i.e. ejaculate) found in the spermathecae of females paired with males <95 mm resulted from mating with those particular males. In laboratory tanks, however, we observed small males to copulate with multiparous females (Ennis et al. 1988a) and there is no reason to suspect such does not also occur in the wild. Also, eight diver-caught pairs that were collected in Bonne Bay in June 1988 were held separately in an on-site seawater system. The males ranged from 66 to 85 mm CW ($\bar{X}$ = 78). After hatching old eggs, each female extruded a clutch of new eggs. There were no signs of egg mortality and all females carried well-developed eggs when observations ceased in late October.

In part three under this heading, Elner and Beninger stated that in order for our conclusion regarding sublegal males being able to substitute for commercial males in normal mating activity to be tenable, the number of eggs extruded by a female which had copulated with a small male would have to be equal to that extruded by a female which had copulated with a commercial-size male. We previously cited Adams and Paul (1983) who found that the number of fertilized eggs extruded by primiparous female *C. bairdi* was independent of male parent size. In addition,Paul (1984) found that the estimated number of eggs per clutch was similar for multiparous *C. bairdi* which mated just prior to egg extrusion and for females forced to use stored sperm to fertilize one egg clutch in captivity, and, compared to the latter, the number of eggs carried by females using stored sperm to fertilize a second successive egg clutch declined by 25%.

This latter comment might be more relevant to the second of our two main conclusions: that population reproductive potential is maintained at a high level despite high exploitation rates on males ≥95 mm. We consider maximum population reproductive potential to mean all mature females in the population at any particular point in time (outside the hatching and egg laying seasons) carrying full clutches of fertilized eggs. Reproductive potential (i.e. annual production of fertilized eggs) could be reduced substantially below maximum and still be considered high. After years of high exploitation rates in productive (i.e. strong annual recruitment) crab fishing areas, the evidence indicates that population reproductive potential is being maintained near its maximum. Compare this, for example, with the situation in the lobster (*Homarus americanus*) fishery where annual egg production is only a small fraction of the maximum population reproductive potential because females are harvested at high exploitation rates at size limits smaller than the sizes at which most females mature.

5. Unfounded General Conclusion

The final sentence of the Discussion in Ennis et al. (1988a) reads: "From the available evidence, we conclude that production of viable eggs, even in heavily fished *C. opilio* stocks, is maintained to the right of the ascending limb of a stock-recruitment curve whatever its general shape might be for this species". In no way did we suggest or imply that this was derived from our results. It followed from our preceding conclusions, which were based largely on our results, as well as our consideration of the relevant literature. Also, we in no way, implicitly or otherwise, equate eggs with recruits as Elner and Beninger state. This statement indicates a lack of understanding on their part of what is meant by a stock-recruitment curve. The ascending limb of a stock-recruitment curve is that portion where recruitment increases in relation to increasing stock. In a stock-recruitment relationship, stock means spawning biomass, abundance of egg-bearing females, total egg production, etc., and recruitment means the resulting number of new animals that will enter the stock (overall population) at some time in the future.

There are only two stock-recruitment curves for large decapod crustacea available in the literature. One is for the western rock lobster (*Panulirus cygnus*) (Morgan et al. 1982) the other is for the red king crab (*Paralithodes camtschatica*) (Jamieson 1986). Both are of the Ricker type, i.e. a long tail or descending limb to the right of the peak. This means that recruitment is low when reproductive potential (i.e. annual egg production) is high.

The snow crab fishery in Atlantic Canada harvests males ≥95 mm only. Females are not only unattractive for harvest but are also not usually retained in commercial traps in significant numbers. As far as is known, the fishery does not have a negative impact on the abundance of females and any fluctuation in their abundance is presumably due to natural causes. Whatever the general shape of the stock-recruitment curve in *C. opilio* might be, there presumably is very wide scope for fluctuation in annual egg production to the right of the ascending limb within which it probably falls.

We indicated in Ennis et al. (1988a) there was no reason to suspect that recruitment to the snow crab fishery in Atlantic Canada would be adversely affected if reproductive potential were not maintained near its maximum. In fact, we considered it reasonable to suggest that recruitment might be increased if reproductive potential were reduced. There are major fluctuations in recruitment in the male-only *Cancer magister* fishery and Botsford's (1986) analyses indicate that there would be less variability if females were also harvested. Further work by Botsford (pers. comm.) also indicates that having egg production too high can lead to recruitment being lower and leaves a population more sensitive to random environmental influences on recruitment, especially when the stock-recruitment relationship is of the Ricker type and females are differentially protected from harvest.

Conclusions

That reproducton in *C. opilio* is complex is obvious and many details remain to be resolved by way of clearly focused research. We are confident, however, that the two main conclusions we reached in Ennis et al. (1988a), regarding sublegal males being able to substitute for larger commercial-size males in normal mating activity and that reproductive potential will be maintained at a high level despite high exploitation rates, were and continue to be well founded. We do not disagree, however, in the case of our final conclusion regarding egg production, even in heavily fished *C. opilio* stocks, being maintained to the right of the ascending limb of a stock-recruitment curve, that "conclude" may have been too strong a term. We believe, however, that the available evidence strongly supports such a conclusion.

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